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A Study of the Ectotrophic Mycorrhizas of Woody Plants.

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With Plates VII—XI and 93 Text-figures.

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INTRODUCTION.

The study of the mycorrhiza has become active since Frank (1885) published his first paper on the subject. He demonstrated the true morphological nature of the ectotrophic mycorrhiza and gave the name to it. He also advanced a hypothesis that it is a symbiotic association of the fungus and the root of a vascular plant, the latter obtaining not only water and mineral salts but also organic substance directly from the former. As to the nature of the mycorrhizal fungus, the same author (1888) maintained that its proper nutrition depends upon soil constituents and not upon the substance of the roots. In fact he was the first who attempted to show the biological nature of the ectotrophic mycorrhizas.

Frank's idea of symbiosis was more elaborately presented in 1900 by Stahl. He stated, after careful observation on a great number of plants, that, in humous soil a severe struggle for essential nutrient salts is going on among numerous micro-organisms and the roots of many vascular plants, and the mycorrhiza-producing plants can obtain the necessary amount of food substances only by the aid of the mycorrhizal fungi which have more advantage in a struggle than the roots of higher plants.

Tubeuf (1903), Sarouw (1903), Möller (1903), and Rexhausen (1920) have supported Frank's idea.

Melin (1921—1925) has carried out histological work on the natural mycorrhizas caused by *Boletus*, and studied the physiological relation between several mycorrhiza-producing plants and fungi in pure culture, coming to the conclusion: "Die Mykorrhizen sind auf Rohhumusböden sehr günstige stickstoffvermittelnde Organe, da die Pilzsymbionten eben so wie die anderen Bodenpilze leicht Ammoniak und organische N-Verbindungen assimilieren können. Infolge der Mykorrhizen kann demnach der Stickstoffbedarf der Bäume trotz der harten Konkurrenz mit den Bodenorganismen gedeckt werden." Thus he is in favour of the theory of Frank and Stahl.

As to the biological significance of the ectotrophic mycorrhizas, there

exists however another idea, namely, the theory of parasitic relation. Abandoning the theory of the symbiotic relation, Woronin (1885) stated the opinion that the envelopment of the roots by the fungous mantle should be taken as a kind of parasitic phenomenon. This theory has been supported by Fuchs (1911), Weyland (1912), McDougall (1914, 1922) and Masui (1926). Fairly good evidence for the theory has been presented by these authors, though more extensive work would be needed to make the theory unshakeable.

In short, although many investigators have attempted to verify or refute Frank's idea, presenting good evidence or ingenous hypotheses pro and con, the true biological nature of the ectotrophic mycorrhizas in general still remains to be elucidated.

Turning to the other side, the systematic position of the mycorrhiza-producing fungi has hitherto been taken into consideration by many investigators. Since the work of Noak the following fungi have been determined to be mycorrhiza formers:

Noak (1889): *Geaster fimbriatus* on *Pinus silvestris* and *Abies excelsa*.
G. fornicatus on do. and do.
Agaricus (Tricholoma) russula on *Fagus*.
Agaricus terreus on *Pinus* and *Fagus*.
Lactarius piperatus on *Fagus* and *Quercus*.
Cortinarius callisteus on *Picea*.
C. coerulescens on *Fagus*.
C. fulmineus on *Quercus*.

Rees and Fisch (1887):
Elaphomyces granulatus on *Pinus silvestris*.
E. variegatus on do.

Pennington (1908):
Russula emetica on *Quercus*.
Tricholoma transmutans on do.

McDougall (1914, 1922):
Russula sp. on *Tilia americana*.
Cortinarius sp. on *Betula papyrifera*.

Boletus scaber v. *fuscus* on do.
Scleroderma vulgare on *Quercus alba*.
Cortinarius sp. on *Abies balsamea* and *Picca rubra*.

MIMURA (1915) : *Cortinellus edodes (Armillaria Matsudake)* on *Pinus densiflora*.

MELIN (1921–25) : *Boletus badius* on *Pinus silvestris*.
B. edulis on *Betula*.
B. elegans on *Larix europaea* and *L. occidentalis*.
B. granulatus on *Pinus silvestris* and *P. montana*.
B. luteus on *B. silvestris*, *P. montana* and *Larix europaea*.
B. rufus on *Betula* and *Populus*.
B. scaber on do. and do.
Amanita muscaria on *Betula*, *Larix*, *P. silvestris* and *Picca Abies*.
Cortinarius camphoratus on *Larix*.
C. mucosus on *P. silvestris* and *P. montana*.
C. balteatus on *Picca Abies*.
Lactarius deliciosus on *P. montana*, *P. silvestris* and *Picca Abies*.
Russula fragilis on *P. montana* and *P. silvestris*.
Tricholoma psammopus on *Larix*.
T. vilgatum on *P. montana*.
T. flavobrunneum on *Betula*.
(MELIN has moreover stated that *Cantharellus*, *Gomphidius*, *Inocybe*, *Hydnum* and *Hygrophorus* seem to be mycorrhiza formers.)

MASUI (1926) : *Cantharellus floccosus* on *Abies firma* and *A. Mayriana*.
Boletus luteus (?) on *Quercus pausidentata*.
Cortinarius sp. (a) on *Alnus japonica*.

Thus almost all of the mycorrhiza-forming fungi hitherto discovered belong to *Hymenomycetes*. This number will undoubtedly be augumented considerably by further investigntions, and MELIN's statement, that

"– vielleicht die meisten von den Humus-Hymenomyzeten der Wälder als Mykorrhiza-bildner herausstellen werden", will possibly be found in future to be a statement of fact.

This paper deals chiefly with (1) the ecological and morphological relation between the mycorrhizal fungi and the roots of the mycorrhiza-producing vascular plants, (2) the systematic position of the mycorrhizal fungi and (3) the biological significance of the ectotrophic mycorrhizas.

1. MORPHOLOGICAL AND ECOLOGICAL RELATION BETWEEN MYCORRHIZA-FORMERS AND ROOTS OF VASCULAR PLANTS.

The present study was made upon material chiefly found in the vicinity of Kyoto.

It has hitherto been considered by the investigators that the fixation of mycorrhizas is difficult. Flemming's solution is clearly unsuitable. Juel's chromo-acetic-platic chloride solution and Zenker's mixture, both of which were introduced by Melin (1923), and chromo-acetic solution are, in many cases, suitable. Besides, Licent's chromo-acetic-formalin mixture was found to be an excellent fixative for the fungous mantle as well as for the cell-contents of the root-tissue.

For staining, aniline blue, dissolved in 3 % solution of acetic acid, or aqueous solution of acid fuchsin were used with good results. Besides these solutions, sometimes, vesuvin-aniline blue, Delafield's haematoxylin or Flemming's safranine-gentian violet-orange were used.

A. Newly found Mycorrhiza-producing Fungi and Their Host Plants.

Although a considerable number of mycorrhiza-formers have been described in Europe and America, those found in Japan are yet very few. In my previous paper (1926, 1, 3 and 4), I described *Cantharellus floccosus*, *Boletus luteus* (?) and *Cortinarius sp. (a)* as mycorrhiza-formers. Since

then I have found 10 species of mycorrhizal fungi, which I may mention here as follows :

Armillaria caligata on *Pinus densiflora*.
Boletus bovinus on *P. densiflora*.
Cortinarius cinnamomeus on *P. densiflora* and *Populus tremula var. villosa*.
C. sp. (a) on *Castanea pubinervis* Schn.
C. sp. (p) on *P. densiflora*.
C. sp. (k) on *P. densiflora*.
Cantharellus floccosus on *P. densiflora*.
Hydnum affine on *P. densiflora*.
Polyporus leucomelas on *P. densiflora*.
Scleroderma vulgare (?) on *Castanea pubinervis* Schn.

1. *Armillaria caligata and its mycorrhiza with Pinus densiflora.*

a. *Armillaria caligata*. The fruiting bodies grow on the ground in pine woods. They occur, in the vicinity of Kyoto, during the latter part of autumn, and are especially abundant in November and December.

b. *Mycorrhizas caused by Armillaria caligata*. The mycorrhizas of *Pinus densiflora* caused by *Armillaria caligata* are found in great numbers beneath the fruiting bodies. They are very small, clavate, 0·2–0·36 mm.

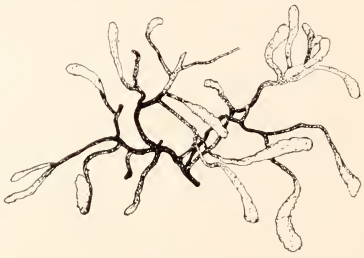

Fig. 1. Mycorrhizas of *Pinus densiflora* caused by *Armillaria caligata*. × ca 7·5.

in diam., enveloped with white mycelium (Text-fig. 1 and Pl. VIII, Fig. 1).

When young, they are white with short projecting hyphae, while those in middle-age are slightly darker, enveloped with densely interwoven thick mycelium especially around their apical portions. With age they turn dark and almost lose their envelope.

Microtomic section of the mycorrhiza clearly reveals its internal structure. The fungous mantle is 7–22 μ in thickness, composed of rather loosely interwoven thin filaments measuring 1·5–2·7 μ in diam., and presents numerous projecting hyphae on its surface.

Beneath the mantle there are usually two rows of depressed cells with large quantity of tannic substance. HARTIG's network develops well between them, but no fungous filaments are found within the tannins.

The second layer is made up of one row of large cells enveloped by HARTIG's network. Interesting things about these cells are that (1) the

Fig. 2. Cross section of a mycorrhiza of *Pinus densiflora* caused by *Armillaria caligata*. × 561.

Fig. 3. Seedlings of *Pinus densiflora* whose roots have been heavily infected by *Armillaria caligata*. × ca 4.

protoplasmic membranes are cut up into granular bodies, 1—4 μ in diam., arranged along the cell-walls and (2) the nuclear structure becomes obscure and nucleoli are lost. These facts seem to indicate that these cells have been heavily injured by the fungous infection, no such phenomenon being found in the cells of the corresponding portion of the normal root.

The next row of the cortex also is composed of large cells but without Hartig's network between them. They show enfeebled appearance in structure like those of the former layer. Sometimes the fungous hyphae enter into the cell-cavities, but they are not digested away by the host cell (Text-fig. 2).

The endodermal cells have a large amount of tannic substance.

When the infection spreads to the growing large roots, they are ultimately killed. The parasitic nature of the fungus is also fully exhibited on roots of seedlings (Text-fig. 3).

c. *Soil-mycelium.* All the mycorrhizas found below the fruiting bodies have the above mentioned characteristics, indicating that they have been caused by one and the same fungus. They are found closely associated and bound together by numerous hyphae projected from each one into a mass forming a layer of soil-mycelium (Text-fig. 4). A vertical section of the soil-mycelium shows the following three parts, beginning at the upper: (1) a mycelial layer, (2) a mycorrhizal layer and (3) a submycorrhizal layer. The mycorrhizal layer is composed of closely associated fresh mycorrhizas and a mycelial network filling up their interspaces. The intercalar mycelium extends upward, forming a continuous thin layer of mycelium over this layer. The third layer is made up of three elements: dark-coloured old mycorrhizas, numerous thin roots or stalks of the mycorrhizas and remains of decayed mycelium. These layers contain a small amount of humous particles within them.

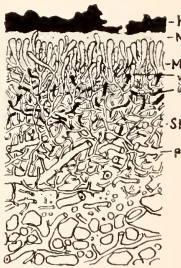

Fig. 4. Diagrammatic representation of a vertical section of the soil-mycelium. *H*, humus; *M*, mycelial layer; *MR*, mycorrhizal layer; *ym*, young mycorrhiza; *om*, old mycorrhiza; *SM*, submycorrhizal layer; *R*, root of *Pinus densiflora*.

Over the soil-mycelium there is a thin layer of rotten leaves, lichens or mosses, which may be of use as a protection against excessive drying

of the mycelium. This layer is easily removable from the mycelial surface, almost no hyphal connection being found between them, indicating that perhaps the mycelium owes little of its food substances directly to the humus.

Most of the mycorrhizas are killed in winter by the infecting fungus, inducing the fade of the soil-mycelium. But there is no time of year when no fresh mycorrhiza can be found in the mycelium, since the formation of new mycorrhizas usually begins before all of the old ones are dead, as described by McDougall (1914). In autumn, when new roots become plentiful in the upper layer of the soil-mycelium of the previous year, they are infected by the mycelium of the old mycorrhizas and are all transformed into new mycorrhizas. Thus, the new mycorrhizal layer is formed over the submycorrhizal layer, namely that of the previous year.

It is a well known fact that soils which contain mycelium are difficult to wet as the mycelium does not easily permit the imbibition of water when once it gets dry. In this case also, the soil-mycelium itself and soils covered by it are very dry.

d. *Mode of the origination of the fruiting body.* In my previous paper I stated that sometimes fruiting bodies of *Cantharellus floccosus* are formed on a mycelial network, which had been interwoven by the hyphae projected from numerous mycorrhizas. Those of *Armillaria caligata* too originate, in the same way, on the mycelium (Pl. IX, Fig. 3). In early September, a considerable number of buttons are found originated on its surface. They are, at first, white, slightly elongated, minute bodies, while they develop into full-sized mushrooms in a few weeks.

An actual connection between the buttons and mycorrhizas being exactly demonstrated under the microscope, it is quite clear that the fungus is one of the mycorrhiza-formers of *Pinus densiflora.*

2. *Armillaria Matsudake (Cortinellus edodes) and its Mycorrhiza with Pinus densiflora.*

a. *Armillaria Matsudake.* The fruiting bodies usually grow in pine

woods during spring and autumn.

It is a well known fact, in Japan, that the living roots of *Pinus densiflora* play an essential rôle in the production of mushrooms. For instance, when pine trees are cut down at some place, no mushrooms occur at that place in the following year. Even if their main roots only are cut, the "matsudake" occur no more on the portion of soil where the rootlets are distributed. These facts almost prove that this mushroom cannot occur except where there are living roots of *Pinus densiflora*.

b. *Mycorrhizas caused by Armillaria Matsudake*. It is very easy to observe the mode of fungous infection on roots in October, a season in which the roots of *Pinus densiflora* are found also in luxuriant growth. Usually the infection upon the young rootlets is attained by fungous

Fig. 5. *Armillaria Matsudake* grown on a soil-mycelium, *M*. *F*, fruiting body; *H*, humous layer.

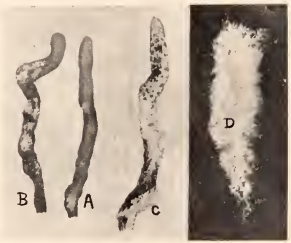

Fig. 6. Mycorrhizas of *Pinus densiflora* caused by *Armillaria Matsudake*. *A–C*, young mycorrhizas; *D*, middle-aged one.

filaments grown from the preexisting mycorrhizas, though on the other hand the hyphae germinated directly from spores may play a part in it. Growing rootlets just infected are still white or yellowish in colour, but the infected portion is more or less discoloured. With the advance of infection all over the rootlets, these turn dark in colour growing no more. Thus the mycorrhizas are completed. Then the hyphae are given off from their surfaces making so luxuriant a growth that they envelope nearly all the apical half of the rootlets with thick mycelial

anastomoses. Such mycorrhizas are found in great number during the middle part of autumn when the mushrooms occur luxuriantly. The mycelial anastomoses thus developed form, by the association of numerous mycorrhiza, a continuous mycelial layer or a soil-mycelium, as in the case of *Armillaria caligata* (Text-figs. 5—6). When the mycorrhizas get old, the mycelium dies away and dark nodose rootlets are left.

A microtomic section of the mycorrhiza shows clearly its internal structure. The fungous mantle is thin, 7—16 μ in thickness, and rather loose in texture. The hyphae given off from its surface are 1·5—3 μ in diam., provided with cross septa. Inserted between the fungous mantle and the cortical tissue there are granular bodies of tannic substance derived from the calyptral cells. The cells of the cortical tissue, when the mycorrhizas are young, have minute or small granular bodies of tannic

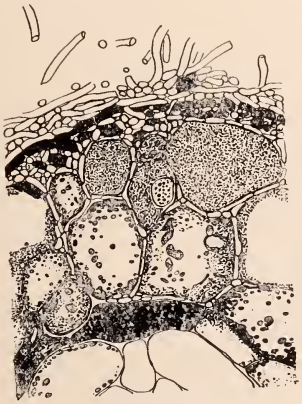

Fig. 7. Cross section of a mycorrhiza of *Pinus densiflora* caused by *Armillaria Matsudake*. × 578.

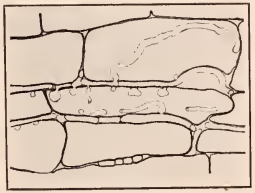

Fig. 8. Longitudinal section of a mycorrhiza of *Pinus densiflora* caused by *Armillaria Matsudake*, showing intracellular hyphae. × 390.

substance filling them up and well-developed intercellular hyphae between them (Text-fig. 7). These cells very much resemble those in the granular layer (Körnerschicht) of the mycorrhiza of *Pinus silvestris* described by Melin (1923). He believes that such granular contents of the cell are substance secreted by the infected fungus. In my opinion they are, however, not the special substance of mycorrhizas, since they are found also in the cells of the normal root. In slightly older ones, usually the

filaments found in the intracellular spaces of the cortical tissue enter into the cell-cavities, dissolving the cell-walls, and form sometimes a well-developed intracellular mycelium (Text-fig, 8). But they are not digested by the host cells. Synthetic investigation shows the same intracellular hyphae in the cortical cells of pine roots (compare p. 200).

c. *Soil-mycelium.* As above mentioned, the soil-mycelium of *Armillaria Matsudake* is produced by the existence of numerous mycorrhizas.

In pine woods, the well-developed soil-mycelium is found usually beneath a thick layer of wood-straw,* 3—10 cm. in thickness, composed mainly of fallen leaves and branches of *Pinus densiflora*. The layer is porous and dampish, and there are found many rootlets of pine usually transformed into coralloid mycorrhizas by various fungi. The mycelium of *Armillaria Matsudake* is formed, however, not in this layer, but beneath it and distinctly separated from it. The physical condition of the soil-mycelium is almost the same as that of *Armillaria caligata*. An important fact about this mycelium is that little of its proper nutrition is obtained from humus, as it contains almost no humus or soil perticles within or not in intimate contact with it.

d. *Mode of the origination of the fruiting body.* During autumn, many primordia of fruiting bodies are produced as minute knots from the mycelium (Pl. IX, Fig. 5). Examination under a good pocket lens or a low-powered microscope reveals that the knots are strictly connected with numerous fresh mycorrhizas, almost irrespectively with humus or dead roots of pine. These facts show that the mycorrhiza is formed by the fungus concerned and the fruiting bodies are produced only at the expense of roots of *Pinus densiflora*.

3. *Boletus bovinus and its mycorrhiza with Pinus densiflora.*

a. *Boletus bovinus.* Several *Boleti* have hitherto been known as

* "Wood-straw" indicates half or almost entirely rotten leaves and branches, found in a piled mass, on the surface of soil in wood. It is dark brown in colour and gives acidic reaction. I have hitherto described (1 and 3, 1926), by mistake, such a thing as "raw humus".

mycorrhizal fungi of vascular plants, but the knowledge that *Boletus bovinus* causes mycorrhizas on *Pinus densiflora* is rather new. The fruiting bodies usually grow on humus or wood-straw in pine woods during spring and autumn. Careful observation under a good pocket lens shown in some cases that they are actually connected with a particular mycorrhiza of the pine (Pl. VIII, Fig. 2), while in other cases no such relation is found. This fact indicates that the fungus may be a facultative mycorrhiza former on *Pinus densiflora.*

b. *Mycorrhizas caused by Boletus bovinus. Boletus bovinus* causes both single and compound mycorrhizas on roots of *Pinus densiflora.* The former are stubby, dichotomously branched, large, 0·3—0·6 mm. in diam., with numerous projecting hyphae or a few bundles of hyphae on the surface (Text-fig. 9). The colour is light yellowish brown

Fig. 9. Mycorrhizas of *Pinus densiflora* caused by *Boletus bovinus.* × 2.5.

when young and fresh, changing to brown with age. The hyphal bundles are given off from the basal portion when the mycorrhiza becomes a little old, and serve for further infection of the newly elongated roots.

The compound mycorrhiza is a cluster of mycorrhizas bound together in one mass by the hyphae given off from their surfaces (Text-fig. 9, D).

Besides the mycorrhizas it contains also numerous soil or humous

particles within it, indicating that it is developed from a single mycorrhiza, increasing in number and size by repeated branching, like that of *Pinus montana* described by MÜLLER (1903).

The fungus infects not only side-branches, transforming them into normal small mycorrhizas, but also growing large roots. In this latter

Fig. 10. A heaviiy infected large root of *Pinus densiflora*. As its apex has been killed by the fungus, a side-branch has begun to grow out. × ca 5.

case the roots are transformed sometimes into large mycorrhizas of the same form as the smaller ones, but sometimes they are killed by the fungus without presenting the stubby form. Frequently there appears a side root from the dead main root (Text-fig. 10) as in the case of *Abies firma*

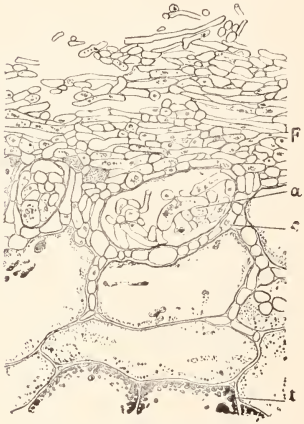

Fig. 11. Longitudinal section of a mycorrhiza of *Pinus densiflora* caused by *Boletus bovinus*. *F*, fungous mantle; *a*, intracellular mycelium; *c*, intercellular mycelium; *t*, tannic substance. × 578.

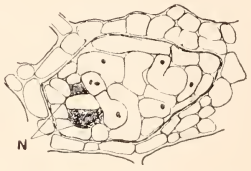

Fig. 12. A cortical cell of the mycorrhiza, filled up by intracellular filaments, showing its nucleus, *N*, divided into two. × 780.

Fig. 13. An amoeboid nucleus found in a cortical cell of the mycorrhiza. × 1300.

previously described by the author (1926, 1, p. 45).

The microtomic sections of the compound mycorrhiza show that it is composed of many ecto-endotrophic mycorrhizas. Its mantle is very thick, 40—60 μ in thickness, differentiating into two parts. The peripheral portion is made up of loosely interwoven thin filaments, 1·5—2·5 μ in diam., giving filaments off outwards, while the inner portion is of closely associated larger ones, measuring 1·5—4·0 μ in diameter. From the inner part of each mantle, a number of large filaments grow inwards between the cortical cells forming an extraordinarily-developed HARTIG's network. Moreover the filaments enter into the cell-cavity dissolving the cell-wall, and form endotrophic filaments (Text-fig. 11). They develop well in the cavity sometimes filling it up (Text-fig. 12).

The fungous cell in young mycorrhizas looks very healthy and is provided with two nuclei and minute plasmic granules which are easily coloured with acid fuchsin.

The cortical cells of the host root appear, on the contrary, much enfeebled through this serious infection. A most remarkable thing that goes on in the cells is a transformation or disappearance of cell nuclei. For instance, some are divided into two or more by the infecting filaments or transformed into an amoeboid form, (Text-figs. 12 and 13), some lose an affinity for stain and some entirely disappear.

On the other hand, degeneration of intracellular filaments

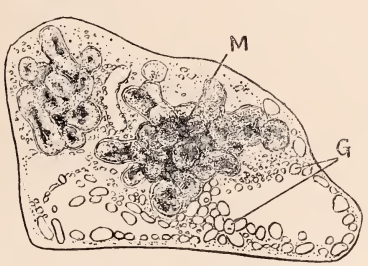

Fig. 14. Slightly degenerated endotrophic mycelium, *M*; *G*, tannic substance. ×1300.

gradually goes on in mycorrhizas at a far advanced stage of development. At first they are imbedded within mucilagenous substances mixed with granules of tannic substances, and then cut up into pieces (Text-fig. 14). As the transformation proceeds, the cell membrane of the hyphae gradually granulates until it does not take the staining dye, although its nuclear substance long retains its capacity for staining (Text-fig. 15). Perhaps such a transformation of fungous filaments in the host cell has been known as "digestion".

Fig. 15. Seriously transformed endotrophic mycelium, *M.* ×1500.

When many large pine roots are found growing within the mycelial beds of *Boletus bovinus*, they are seen microscopically to be all infected by the fungus, even though some of them appear, to the naked eyes, to be free from the mycelium. The fungous filaments advance along the surface of the root entering directly into or between the cortical cells. Thus the infecting

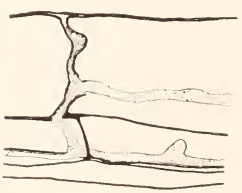

Fig. 16. A longitudinal section of an infected large root, showing intracellularly infecting filaments. ×520.

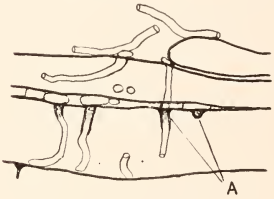

Fig. 17. A longitudinal section of an infected large root, showing intracellularly infecting hyphae partly covered with membraneous substance, *A.* ×692.

filaments diverge, sending branches in every direction through the cell-wall of the host cell, until they kill the root (Text-fig. 16). As soon as the filaments enter the cell cavity, they are partly covered with thick membraneous substances as shown in the figure (Text-fig. 17). *Boletus bovinus*

shows in this case the parasitic nature decidedly.

c. *Mycelium.* Mycelium of *Boletus bovinus* is usually found in a superficial layer of humous soil or wood-straw. It develops sometimes diffusedly or forming complicated hyphal bundles without actual connection with roots of *Pinus densiflora*, while frequently it extends, from the mycorrhizal mass, into the surrounding soil.

4. *Cortinarius cinnamomeus and its mycorrhizas with Pinus densiflora and Populus tremula var. villosa.*

a. *Cortinarius cinnamomeus.* A species of *Cortinarius* which grows in the vicinity of Kyoto actually connected with mycorrhizas of *Pinus densiflora* and *Populus tremula var. villosa*, was decided to be *Cortinarius cinnamomeus* from the following characteristics:— It is a pretty fungus 3—6 cm. high, the cap 2—5 cm. broad and the stem 4—6 mm. thick. The pileus is conical, or convex, and nearly expanded, sometimes nearly plane, and again with a prominent blunt or conical umbo. The surface is smooth, silky, with innate fibrils, and often there are concentric rows of minute scales near the margin. The colour of the pileus is yellow ochre or ochraceous tawny. The gills are adnate, and slightly sinuate, and their colour is sometimes the same as that of the pileus, sometimes buck horn brown or russet colour. The stem is rather slender, fibrous, and in colour the same as the pileus. The flesh is light buff or yellowish brown. The spores are brownish yellow, elliptical and $4{\cdot}9—6{\cdot}5 \times 7{\cdot}1—9{\cdot}0\ \mu$.

So far as I know, *Cortinarius cinnamomeus* has not yet been described as a mycorrhiza former, even though many species of this genus have been considered to be so.

b. (1). *Mycorrhiza of Pinus densiflora caused by Cortinarius cinnamomeus.*

It is 0·4—0·5 mm. in thickness and yellowish in colour, when young and fresh, changing to brown or dark with age. It branches dichotomously giving off many bundles of hyphae from its surface (Pl. X, Fig. 7). Usually the bundles taper off branching into smaller bundles or filaments

and spread upon rotten leaves or trunks. The hyphae found in wood-straw are large, 1·5—5·0 μ in diam., and yellowish in colour, provided with clump-connections (Text-fig. 18).

Microtomic sections of the mycorrhiza show a rather thin fungous

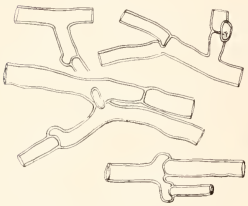

Fig. 18. Hyphae of *Cortinarius cinnamomeus*. ×650.

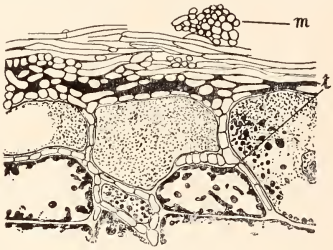

Fig. 19. Section of a mycorrhiza of *Pinus densiflora* caused by *Cortinarius cinnamomeus*. *m*, mantle; *t*, a bundle of filaments; *t*, tannic substance. ×578.

mantle, 7—20 μ in thickness, and well-developed HARTIG's network. The cells of the cortical tissue have a large amount of minute or small granules (Text-fig. 19).

The fungus not only transforms the side-branches into normal mycorrhizas, but also infects large growing roots of the pine. When large roots grow through the mycelial beds of this fungus, they are usually infected by it. Examination with a low-powered microscope reveals yellowish filaments or bundles of filaments running along their surface. The filaments enter directly into the host cells, forming intracellular filaments (Text-fig. 20). They develop further in the cortical tissue, fully presenting the parasitic nature.

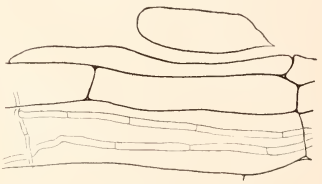

Fig. 20. Intracellular filaments of *Cortinarius cinnamomeus* found in a cortical cell of a large root. ×433.

(2). *Mycorrhizas of Populus tremula var. villosa caused by Cortinarius cinnamomeus.* They are 0·18—0·27 mm., and pale yellow-orange

or pale orange-yellow in colour. The characteristics of the bundles of filaments, given off from their surfaces, are just the same as in the former case (Pl. X, Fig. 4). They have a rather loosely interwoven thin fungous mantle, measuring 15—39 μ in thickness, and well-developed HARTIG's network between the obliquely elongated epidermal cells. Frequently intracellular filaments are found in these cells, but they do not seem to be digested. The host cells contain large quantities of tannic substance (Text-fig. 21).

Fig. 21. Longitudinal section of a mycorrhiza of *Populus tremula var. villosa* caused by *C. cinnamomeus*. *t*, tannic substance. ×578.

The fresh mycorrhizas are found plentifully in early spring perhaps in accordance with the luxuriant growth of roots. In this period, fungous infection is violently carried on not olny on young side-branches but also on growing large roots. They are infected in this case chiefly by a mycelium elongated from preexisting neighbouring mycorrhizas (Pl. X, Fig. 3).

The infected mycelium advances, enveloping the root and giving off yellowish bundles of filaments into the surrounding soil, from the infected portion towards the apex of the root (Pl. X, Fig. 5).

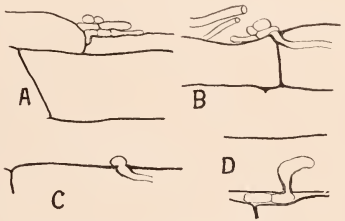

Fig. 22. Longitudinal section of roots of *Populus tremula var. villosa*, showing mode of infection of fungous filaments. *A*, a filament pushing its way between the epidermal cells; *B*, and *C*, filaments that have entered an epidermal cell; *D*, intercellular filament that has entered a cortical cell. ×780.

Microtomic sections show

that even the young white root just growing, which still lacks the ordinal mantle, is infected more or less by the fungus. Fungous filaments elongated along the young root enter direct into the cell-cavity of the epidermal cell through the cell-wall, or first between these cells and then into the cell-cavity of the adjacent cells (Text-fig. 22). When the filaments enter the cavity, they are covered with membraneous substances secreted by the host cell.

The intracellular hyphae are rather thick filaments, 3—4·5 μ in diam., granulated, and seem to show a parasitic nature (Text-fig. 23).

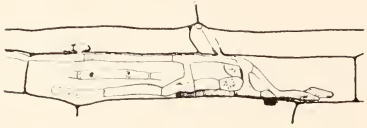

Fig. 23. Longitudinal section of an infected large root of *P. tremula var. villosa*, showing well-developed intracellular mycelium in a cell. ×520.

c. *Mycelium.* The mycelium of *Cortinarius cinnamomeus* grows in the superficial layer of wood-straw or humous soil in connection with living roots of *Pinus densiflora* and *Populus tremula var. villosa*. I have not yet met with a case where it has been formed free from them, even though the mycorrhizas themselves seem to have an intimate relation with humus. These facts indicate that the fungus may be an obligate mycorrhiza former of these plants.

d. *Mode of the origination of the fruiting body.* The bundles of the fungous filaments given off from the surface of the mycorrhizas, serve not only for the further infection of the new roots, but also for the origination of the fruiting body. A few or more bundles elongated on wood-straw unite into one and differentiate into a minute button of the fungus (Pl. VIII, Fig. 4). Specimens which demonstrate such an actual connection, between the mycorrhiza and the mushroom, are easily obtained in the vicinity of Kyoto.

5. *Cortinarius sp. (a) and its mycorrhiza with Castanea pubinervis* Schn.

a. *Mycorrhizas of Castanea pubinervis caused by Cortinarius sp. (a).* *Cortinarius sp. (a)* causes white mycorrhizas not only on roots of *Alnus japonica* (Masui 1926, 4) but also on *Castanea pubinervis*. The mycorrhizas produced in both plants are almost the same in external characteristics, excepting that those of the latter are more or less smaller than the former. Microtomic sections of the mycorrhiza clearly show a rather thin pseudoparenchymatous fungous mantle, 13—20 μ in thickness, made up of thin-walled filaments, 2—3·2 μ in diam., and numerous hairs, with clump-connections, given off from its surface. Beneath the mantle there is a wide layer of epidermal tissue which is composed of obliquely elongated cells with well-developed Hartig's network between them. The epidermal cells have almost no plasmic contents. The protoplasmic contents of the cortical cells are so seriously affected by the chemical stimulation of the fungus that they coagulate, losing the capacity for staining with dye. The endodermis and remains of calyptral cells, found beneath the fungous mantle, have a arge amount of tannic substances (Text-fig. 24).

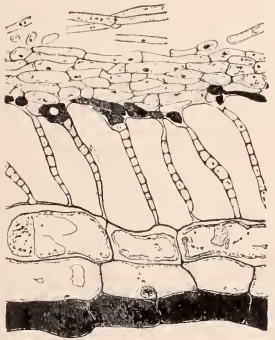

Fig. 24. Longitudinal section of a mycorrhiza of *Castanea pubinervis* caused by *Cortinarius sp. (a)*. ×578.

In respect to the internal structure, the mycorrhiza differs greatly from that of *Alnus japonica* though both mycorrhizas are formed by the same species of *Cortinarius*. The marked difference is that the fungous mantle is rather thin, and has no differentiation of layers as in the case of *Alnus*-mycorrhiza, and moreover it has obliquely elongated epidermal cells, which

are not found in the latter.

b. *Soil-mycelium.* The mycelium is formed only in connection with living roots of *Castanea pubinerbis* as in ordinary cases of the obligate mycorrhiza-formers.

c. *Mode of origination of the fruiting body.* The mode of origination of the fruiting body is just the same as in the case of *Alnus japonica* described formerly (MASUI 4).

6. *Cortinarius sp. (p) and its mycorrhiza with Pinus densiflora.*

a. *Cortinarius sp. (p)* grows in pine woods during autumn. It is a very pretty fungus 2·5—4·5 cm. high, the cap 2—4 cm. broad and the stem is usually bulbous. The pileus is expanded, plane or slightly concave, and its surface is glutinous when moist. The colour of the pileus varies from pale yellow orange to raw sienna or sudan brown. The flesh is light yellowish with strains of light buff. The gills are adnexed or sinuate and the colour of them varies from yellow to rusty. The colour of the stem is the same as that of the pileus or sometimes more brownish. The spores are eliptical, 3·5—5 × 5·7—9·2 μ and brownish yellow in colour (Text-fig. 25).

Fig. 25. *Cortinarius sp. (p).* Natural size.

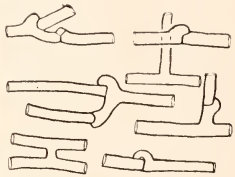

Fig. 26. Hyphae of *Cortinarius sp. (p).* ×780.

b. Mycorrhizas of *Pinus densiflora* caused by this fungus are pure white in colour, and give off numerous hyphae or hyphal bundles from their surfaces (Pl. X, Fig. 2). The hyphae are 1·5—3·0 μ in diam., and provided with clump-connections. Hyphal anastomoses occur occasionally (Text-fig. 26). The mycelium of this fungus is formed only

in connection with the living roots of the pine found in a superficial layer of wood-straw, indicating that the fungus is also an obligate mycorrhiza former (Text-fig. 27). In autumn, the

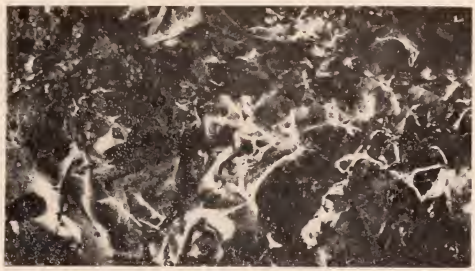

Fig. 27. Mycelium of *Cortinarius sp. (p)*, formed in a superficial layer of wood-straw. × ca 2.

hyphal bundles given off from only one or a few mycorrhizas serve for the formation of white young buttons (Pl. IX, Fig. 2). The actual connection, thus mentioned, was fully demonstrated by many specimens collected in the vicinity of Kyoto and Shiga-Prefecture.

7. *Cortinarius sp. (k) and its mycorrhiza with Pinus densiflora.*

At first I found this fungus at Takayashiro, Gifu-Prefecture on the 1st of November, 1925. After that I found it also at Shôbara, Hiroshima-Prefecture on the 15th of October, 1926. In both cases an actual connection between the fungous fruiting bodies and mycorrhizas was clearly demonstrated.

Fig. 28. *Cortinarius sp. (k)* grown on wood-straw.

a. *Cortinarius sp. (k)*. The plants are 3—7 cm. high, the cap 2—

6 cm. broad, and the stem above is 4—7 mm. in thickness, and below 8—23 mm. in thickness.

The pileus is convex to nearly expanded, and sometimes a little depressed, usually, however, remaining convex at the top. The surface is smooth, more or less silky. The colour is usually pale pinkish buff, sometimes mikado-brown, and darker at the center. The gills are usually sinuate and slightly broader in the middle. The colour of the gills is yellowish brown. The spores are tawny in mass, oval, 4·3—7 × 7—10·6 μ. The stem is clavate, pale pinkish buff in colour, solid, bulbous, and the bulb is often very large.

The veil is prominent, abundant threads are found attached from the upper portion of the stem to the margin of the pileus when young (Text-figs. 28 and 29).

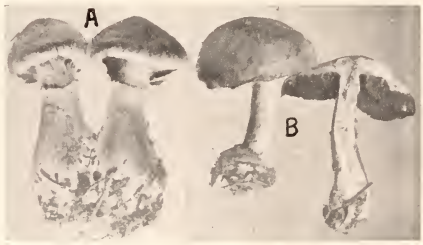

Fig. 29. *Cortinarius sp. (k).* A, young fruiting bodies; B, those in full maturity. A×1; B×¾.

b. *Mycorrhizas of Pinus densiflora caused by Cortinarius sp. (k).* The mycorrhizas are found in clusters in wood-straw in pine woods. They are white with strains of yellow in colour, long, irregularly bent and 0·3—0·36 mm. in diameter. Hyphal bundles are found given off from their surfaces.

c. *Mode of origination of the fruiting body.* Young fruiting bodies of this fungus were found in large numbers among the mycorrhizas. I found, on careful observation, that each button had been formed at the termination of the hyphal bundles. When very young, as just formed,

they are minute white knots. But they turn yellowish gradually (Pl. X, Fig. 6).

8. *Cantharellus floccosus and its mycorrhiza with Pinus densiflora.*

I have already reported that *Cantharellus floccosus* is a mycorrhizal fungus of *Abies* (Masui 1926, 1). On the 16th of October, 1926, I found however the fungus growing in a wood of *Pinus densiflora* at Shôbara, Hiroshima-Prefecture. By careful digging below the mushrooms I found numerous pine roots which had been transformed into mycorrhizas (Text-fig. 30). The mycorrhizas are white, 0·44—0·54 mm. in diam. and give off white hyphae into the surrounding soil, as in the case of *Abies firma* (Pl. X, Fig. 8). In this case the fruiting bodies were orginated only from the mycorrhizal mass.

Fig. 30. *Cantharellus floccosus* growing on ground under a pine tree. ×½

As the soil, in which the mycorrhizas were found, was yellow clay containing scarcely any humous particles, the fruiting body seems to grow chiefly at the expense of the pine root.

9. *Hydnum affine* (Lloyd) *and its mycorrhiza with Pinus densiflora.*

a. *Hydnum affine* (Lloyd). *Hydnum affine*, or *Shishitake*, to give in its local name, was found, in 1923, in a pine wood, during October and November, originated on a mass of mycorrhizas of *Pinus densiflora* (Text-fig. 31). I considered then that the formation of the mushroom might have an intimate relationship with the living roots of it. After three years I was able to decide that it was mycorrhizal fungus of the tree.

Fig. 31. *Hydnum affine* ×⅔ growing on a soil-mycelium.

b. *Mycorrhizas of Pinus densiflora caused by Hydnum affine.* Below the fruiting bodies there are always found numerous particular mycorrhizas with a peculiar smell. They are like those of *Armillaria caligata* in structure, differing from them in smell and also in colour slightly.

In early autumn, when the roots of *Pinus densiflora* grow luxuriantly there are found a considerable number of young roots which have just

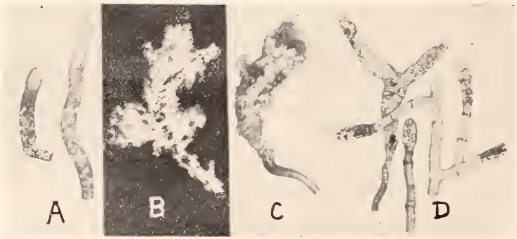

Fig. 32. Mycorrhizas of *Pinus densiflora* caused by *Hydnum affine*, arranged, from *A* to *D*, in their successive stages of development.

been infected. They are growing white roots or rootlets with the basal portion discoloured brown by the infection. When the infected mycelium

develops covering all their surfaces, they elongate no more, turning brown or dark brown. As soon as the mycorrhizas are completed, the fungous mantle makes luxuriant growth enveloping their apical half of the whole length with a very thick network. Those found beneath the fruiting bodies are all in this stage. The mycelium changes first to a yellowish colour with age and then dies out, leaving the dark-coloured rootlets (Text-fig. 32). Through this procedure, numerous pine rootlets are killed.

They have the fungous mantle, 7—29 μ in width, made up of rather loosely associated filaments, 1·5—3·5 μ in diam., and a well-developed intercellular mycelium. Abundant granules of tannic substances are seen in the cells of the cortical tissue.

The mycelium of *Hydnum affine* not only transforms the rootlets of the pine into normal mycorrhizas but also infects large growing

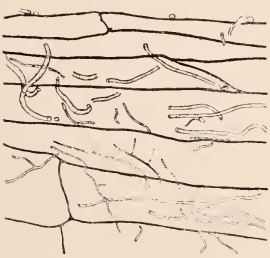

Fig. 33. A longitudinal section of an infected large root, showing intracellularly developed filaments. ×433.

Fig. 34. A heavily infected large root, *A*, from which three side-branches, *B*-*D*, have been formed successively. ×4.6

roots (Text-fig. 34). In this case the roots are mainly infected intracellularly without presenting even a trace of abnormal thickening. Microtomic sections of such roots show abundant filaments

that have entered the cortical cells (Text-fig. 33). When a growing apex of a large root is heavily infected (Text-fig. 34, A), a second root, *B*, comes up as its side-branch, to be killed, and so on. Among large roots of the pine such a replacement of new roots continues within the mycelial layer. The parasitic nature is also fully presented in seedlings of a pine. When seeds germinate on the mycelium, they are fatally infected and killed (Text-fig. 35).

Fig. 35. Seedlings of *Pinus densiflora* germinated on a mycelium of *Hydnum affine*, showing heavily infected roots.

Fig. 36. *Hydnum affine* attached to a mycorrhizal mass. ×1.

c. *The soil-mycelium and the mode of the origination of fruiting bodies.* *Hydnum affine* forms thick mycelium, 3—7 cm. in thickness, in a superficial layer of soil in pine woods. The soil-mycelium is almost completely exposed to the air or covered with a thin layer of fallen leaves. Its vertical section shows, beginning at the upper portion, a mycelial layer, a wide mycorrhizal layer and a submycorrhizal layer, as in the case of *Armillaria caligata*. The second layer is composed of numerous vertically arranged mycorrhizas as its framework and the mycelium which has been interwoven by the hyphae projected from each one (Pl. IX, Fig. 4). The intermycorrhizal mycelium develops upwards forming a thin mycelial layer over this layer. The third layer is a network of abundant pine roots, including old mycorrhizas and remains of the decayed mycelium. The

soil-mycelium is usually dry containing few humous particles.

In early October, when the mycorrhizas show their utmost development, the fruiting body of this fungus originates as a minute knot imbedded in the mycelium, and their connection can be traced cleary.

10. *Polyporus leucomelas and its mycorrhiza with Pinus densiflora.*

a. *Polyporus leucomelas.* The mushroom, "*Kurokawa*" in the local name, grows in pine woods during autumn. It is produced in two ways: the one is on a soil-mycelium formed by numerous mycorrhizas, as in the case of *Armillaria caligata* and *Hydnum affine* (Text-fig. 37), and the other is directly from the infected root as in the case of *Cantharellus flocosus* (Text-fig. 38 and Pl. IX. Fig. 1).

Fig. 37. *Polyporus leucomelas* (×) produced on a soil-mycelium. × ca ¼.

Fig. 38. Several buttons of *Polyporus leucomelas* produced free from the soil-mycelium. × ca ½.

b. *Mycorrhizas and the soil-mycelium.* Mycorrhizas of *Pinus densiflora* caused by *Polyporus*

leucomelas are clavate ones with very long stalks. When young they are slender rootlets, while in middle age the characteristic form is realized. Numerous hyphae being given off from the fungous mantle, ultimately their apical portion is covered up with a thick hyphal network. Such a mycorrhiza is found in large numbers in the superficial layer of soil, and forms networks which unite with one another into a continuous mycelium over the surface of the soil. The fruiting body of this fungus originates, like the first form mentioned above, on such a mycelium. In the old stage of development, the enveloping mycelium of the mycorrhiza dies out, leaving behind a dark coloured nodose root (Text-fig. 39).

Fig. 39. Mycorrhizas of *Pinus densiflora* caused by *Polyporus leucomelas*. *A*×2 ; *B*×6.

b. *Infected roots.* *Polyporus leucomelas* not only transforms young side-branches of *Pinus densiflora* into ectotrophic mycorrhizas but also infects its older roots, enveloping them with a thick mycelium (Pl. X, Fig. 9). A cross section of them shows a very thick enveloping mycelium, 0·09—0·3 mm. in thickness, including soil particles and depressed cortical cells within it. The mycelium of its inner part

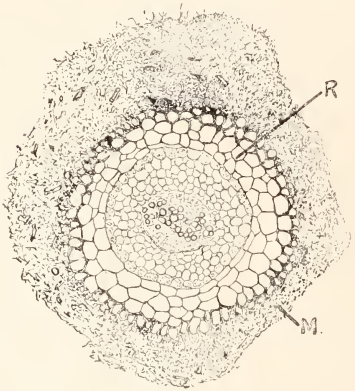

Fig. 40. Cross section of the infected root. *M*, mycelium ; *R*, root of *Pinus densiflora*. ×80.

enters in between the cortical cells, dissolving the middle-lammella, and forms a kind of HARTIG's network, so that the cells of the outermost row of the cortical tissue are isolated as islands within the mycelium (Text-fig. 40).

d. *The origination of the fruiting body.* As mentioned above, occasionally the buttons originate on the side or at the termination of the infected large roots (Text-fig. 42). Sometimes a few or several small

Fig. 41. Two small buttons (*B*) originated on small infected roots. × ca 3.

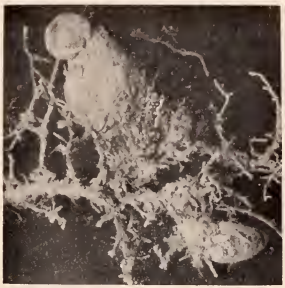

Fig. 42. Two buttons originated on both sides of an infected root. × ca 1.5.

mycorrhizas are concerned in the formation of the minute buttons as shown in the figure (Text-fig. 41). The larger fruiting bodies are usually formed attached to the larger roots and those originated from a few of the small mycorrhizas are usually sterile.

Though a considerable number of species of Basidiomycetes have been hitherto known as mycorrhiza formers, *Polyporus* has not yet attracted the notice of many investigators. It is remarkable moreover, that the mycorrhizal fungus infects such old roots as these and produces fruiting bodies.

11. *Scleroderma vulgare (?) and its mycorrhiza with Castanea pubinervis.*

a. *Scleroderma vulgare* (?). The fungus grows scattered or in small

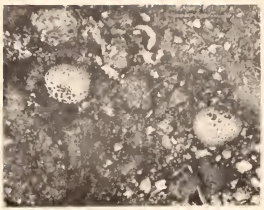

Fig. 43. *Scleroderma vulgare* (?) grown on ground. ×1

groups on the soil under trees (Text-fig. 43). The peridium is rather thick, formed of one layer, and narrowed below into a rooting base. It is subglobose or globoso-depressed, sometimes ovate, and the surface is white when young though in maturity it becomes, greyish, and breaks up into irregular patches. The flesh of it is white when young becoming slightly pinkish when cut. The gleba is

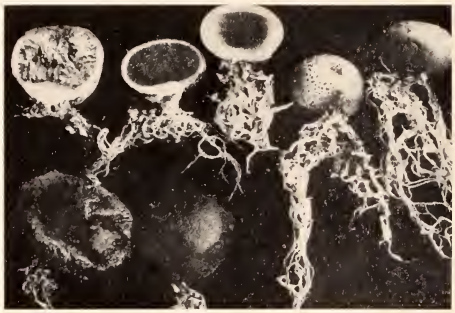

Fig. 44. *Scleroderma vulgare* (?) ×1.

of a purplish dark colour in maturity, and the spores are liberated when the upper portion of the peridium is irregularly split. The spores are spined, spherical, and 10—11 μ in diameter (Text-fig. 44).

b. *Mycorrhizas of Castanea pubinervis caused by Scleroderma vulgare* (?) McDOUGALL in 1914 stated that *Scleroderma vulgare* causes white mycorrhizas on *Quercus*

Fig. 45. Mycorrhizas of *Castanea pubinervis* caused by *Scleroderma vulgare* (?).

Fig. 46. Section of two mycorrhizas of *Castanea pubinervis* caused by *Scleroderma vulgare* (?), showing a hyphal bundle given off from one of them.

alba. Mycorrhizas of *Castanea pubinervis* caused by *Scleroderma vulgare* (?) are rather long, irregularly bent, 0·23—0·39 mm. in diam., and white in colour (Text-figs. 45 and 47). The fungous mantle is thin and the surface is rather smooth, giving off hyphal bundles. The epidermal tissue is composed of anticlinally elongated cells with well-developed intercellular hyphae between them (Text-fig. 47).

The hyphal bundles are found in large numbers associated with the mycorrhizas (Text-fig. 45). They are pure white in colour, giving off numerous hyphae into the surrounding soil. The hyphae are 1·5—4·7 μ in diam., transversely septated and give off branches almost at right angles (Text-fig. 48).

Fig. 47. Hyphal bundles produced from the mycorrhizas

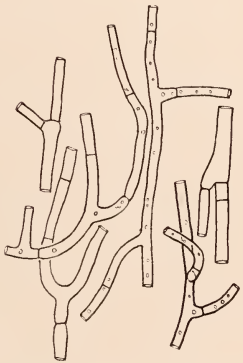

Fig. 48. Hyphae of *Scleroderma vulgare* (?). ×578.

c. *The mode of origination of the fruiting body.* The fungus was grown in large numbers under *Castanea pubinervis* in the yard of this

university during September 1925. I found then by careful digging below them that the hyphal bundles given off from the mycorrhizas are actually connected with the fruiting bodies. In 1926, many specimens showing the same relation were collected also at the same position (Pl. VIII, Fig. 3).

B. General Relation between Mycorrhiza-producing Fungi and their Host Plants.

In the previous chapter I mentioned nine different kinds of mycorrhizas of *Pinus densiflora* and two of *Castanea pubinervis*. The characteristics of these mycorrhizas result, on the one hand, from the specific nature of each fungus concerned, as different fungi may stimulate the roots in different ways, inducing unlike modes of growth. On the other hand *Cortinarius cinnamomeus* and *Cortinarius sp. (a)* cause different mycorrhizas according to the kind of the tree, indicating that the specific nature of the host plant also plays a rôle in its formation, as a different host may respond in an unlike way to the same kind of fungous stimulation. These facts show that the characteristics of each ectotrophic mycorrhiza result from the combination of its two constituents, the host and the fungus, though in many cases the latter is more effective.

The parasitic nature of the mycorrhiza-producing fungi finds sufficient expression in the infected large roots. Such roots are seriously invaded by the infecting fungi until they are ultimately killed.

In the case of the normal mycorrhizas, they are also killed by the fungus within several months, so that it may be designated as "annual." When the infection proceeds to a certain extent, the root grows no more, presenting a clavate or stubby form. The extraordinary growth of the projecting hyphae follows then, especially around its apical portion

where the nutrient is chiefly located. As this behavior is always observable in the mycorrhizas mentioned above, it seems to indicate the parasitic nature of the fungi concerned, though it needs more critical investigations to settle the question.

So far as I am aware, the fruiting bodies of all these mycorrhizal fungi treated in the previous chapter or previous paper may be met with soon after the middle of August, when the mycorrhizas attain their fullest development.

The modes of the origination of the mycorrhiza-producing fungi are distinguished into the following four types:

1. Fruiting bodies originate directly on the infected roots..... *Polyporus leucomelas* and *Cantharellus floccosus*.

2. Fruiting bodies originate, without any connection with humus, on a mycelial network interwoven by the hyphae projecting from numerous mycorrhizas.....*Armillaria caligata*, *A. Matsudake*, *Hydnum affine*, *Polyporus leucomelas* and *Cantharellus floccosus*.

3. Fruiting bodies originate on a few mycelial strands projected directly from the mycorrhizas which are found related with humus..... *Cortinarius cinnamomeus*, *C. sp. (p)* and *C. sp. (k)*.

4. Fruiting bodies originate usually on humus, frequently connected with mycorrhizas.....*Boletus bovinus* and *Scleroderma vulgare* (?).

The mode of the origination of *Cortinarius sp. (a)* stands between 2 and 3.

The behavior of the origination of the mycorrhiza-formers indicates to a certain extent their biological nature. Fungi orginating according to 1, 2 and 3 are obligate mycorrhiza-formers and those in the 4th mode are facultative ones.

C. Morphological Study of Pine Mycorrhizas Caused by Unknown Fungi.

1. *Cluster-type mycorrhiza of Pinus densiflora.*

In order to know the morphological nature of the mycorrhizal root,

it is also necessary to study the normal root and compare the difference between them.

a. *Normal root.* Materials used for the present purpose were obtained from pines cultivated on sterilized soil in ERLENMEYER-flasks as well as from those grown naturally.

The meristematic portion of the uninfected root is made up of cells with large nuclei, 9–14 μ in diam., and rich in cytoplasm. The nucleus comprizes a fine nuclear reticulum composed of karyotin and two or more nucleoli, measuring 1–3 μ in diameter. Nuclear divisions occur very frequently. These cells contain no conspicuous ergastic inclusions within the cytoplasm, and are separated by delicate walls with no intercellular spaces (Pl. VII, Fig. 1).

As the cells move from the region of greatest meristematic activity, they become diversified in structure in connection with their specialization in function. In young cells of the periblem, tannic substance begin to appear, in granules or more or less elongated bodies measuring 0·5–1·5 μ in diam., in small vacuoles formed around the nucleus (Pl. VII, Fig. 2). Such bodies frequently seem as if they were pieces of fungous filaments. In some cases tannic substances are deposited in numerous minute granules within the vacuoles as shown in the figure (Pl. VII, Fig. 3).

At a short distance from the meristematic portion, tolerably elongated cells begin to show large vacuoles with distinct vacuolar-membranes in the cytoplasm. The tannic granules increase in number, sometimes filling up the vacuoles (Pl. VII, Fig. 4).

The fully developed cell of the cortex is provided with a thin peripheral layer of cytoplasm, a nucleus with distinct nucleolus, and a large central vacuole which comprizes abundant granules, 1·5–3·2 μ in diam. (Pl. VII, Fig. 6), or minute granules filling it up as shown in the figure (Pl. VII, Fig. 5). Cells of the root-cap and outermost row of the cortex usually contain masses of tannins.

When chromo-acetic solution or chromo-acetic-platic chloride solution is used for the fixation, the tannic substances found in the cells of the

peripheral region, namely, two or three rows of the outer cortex, are coloured green or light green with aniline blue and reddish brown or light reddish brown with FLEMMING's triple staining; while the tannic granules in the inner region do not take any staining dye, perhaps as these bodies are fixed only by acetic acid which penetrates sooner than the chromic acid. The tannic substance in cells of the inner cortex is, therefore, hyaline or more or less yellowish, maintaining characteristic granules measuring 1·5–3·2 μ in diameter.

Fig. 49. A seedling of *Pinus densiflora* with numerous mycorrhizas on its roots. ×ca 1.3.

b. *Mycorrhizas.* This mycorrhiza resembles the cluster-type ("Strauss- Typus") mycorrhiza described by MELIN (1923). It is 0·28–0·32 mm. in thickness and branches dichotomously. The colour of it is very light brown or light brown, changing to brown or dark brown with age (Text-fig. 49).

The fungous mantle is thin, measuring 8–20 μ in width, made up of hyphae, 2·2–3·1 μ in diameter.

The meristematic portion of the mycorrhizal root is made up of cells with very large nuclei, 12–19 μ in diam., and degenerated cells inserted between them. The former contain no conspicuous ergastic inclusions or fungous filaments within the cytoplasm which is less compact than that of the normal root, and are separated by very delicate walls with neither intercellular spaces nor HARTIG's network. The nucleus consists of a rougher nuclear reticulum, than that of the normal root, and is frequently devoid of a nucleolus. In rare cases, nuclear division occurs. The degenerated

cell contains an irregularly shaped dark body (dead nucleus?) in the central portion (Pl. VII, Fig. 8).

There are a few or more cell-rows between the meristematic portion and the fungous mantle (Pl. VII, Fig. 7). The outermost row, which is in contact with fungous mantle, is made up of seriously depressed cells with dark substances. The cells of the second row contain homogeneous tannins filling up the cell cavities. They contain no intracellular hyphae and no Harting's network between them. Those of the third or fourth row are provided with a large nucleus with no nucleolus, and granular bodies of tannic substances within the vacuoles which have distinct vacuole-membranes (Pl. VII, Fig. 9).

At portions distant 30–50 μ from the meristematic region, the cells of the periblem already show themselves much inflicted by the infecting fungus. The thick vacuolar membrane or tonoplast becomes wavy, seriously separated from the cytoplasm, and the nucleus, which is 7–11 μ in diam., is entirely devoid of the nucleolus.

Fully-developed cortical cells enveloped or half enveloped by the Hartig's network contain massive or granular bodies, measuring 1·5–3·2 μ in diam., of tannic substance in the central vacuoles, forming a tannic sheath (Gerbstoffscheide) and granular layer (Körnerschicht) described by Melin (Pl. VII, Figs. 10–11). It is clear that these intravacuolar substances are not the special ones of the mycorrhiza, as they are found also in the uninfected roots. The nuclear structure becomes indistinct, forming a vague nuclear reticulum or dull granules, indicating much decreased nuclear activity (Pl. VII, Figs. 11–13).

The cells of the inner cortex contain so-called hyaline granules which are found also in the corresponding portion of the normal root. There are neither fungous hyphae in the cells of the tannin sheath nor those which are digested away by the host cells.

I have frequently observed, on granitic sand soil in autumn, that vigorous young pines with dark green leaves usually have a larger

number of fresh mycorrhizas on their roots than feeble ones with yellowish leaves. A similar example has been also mentioned by MELIN (1923). He believes this to be merely an evidence that pines are benefited by mycorrhizas, but, in my opinion, an alternative is also possible, namely, that a prominent development of fresh mycorrhizas in autumn is seen only on roots of vigorous plants because these produce abundant new roots.

2. *Mycorrhizas of Pinus silvestris.*

I found two kinds of mycorrhizas, Forms A and B, on the roots of *Pinus silvestris* grown on soil in the school yard of this university.

Form A.

This mycorrhiza resembles the cluster-type mycorrhiza described by MELIN (1923). It is 0·25–0·41 mm. in thickness and sends out branchlets dichotomously sometimes forming a small tuft by the repeated branching. The colour of it is light brown or yellowish brown, changing to brown or dark brown with age.

The fungous mantle is rather thin, 7–29 μ in thickness, made up of thin hyphae, 1·5–2·0 μ in diam. There is a row of depressed cells with much tannic substances within them isolated from the root-tissue. This layer may correspond to the outer tannic sheath (äussere Gerbstoffscheide) described by MELIN. He reports that very thin hyphae which have been denoted "haustorial hyphae" are constantly found imbedded in the tannins; but in this case the existence of such hyphae is quite rare.

The cells of the meristematic portion of the cortex contain large nuclei and are rich in plasmic substance, both of which are coloured blue with aniline blue. The nucleus provides a few or more nucleoli. These cells are entirely lacking in fungous filaments as well as in a HARTIG's network between them, though MELIN found a very thin non-septated endophyte. When the cells become a little larger, several small vacuoles are formed around the nucleus and tannic substances are secreted in them. As the development advances, the vacuoles become larger uniting together and then the vacuolar membrane, which has faint affinity

for any staining dyes, almost adheres to the inner surface of the cell-wall. The intercellular hyphae develop between such cells. As soon as the cells are enveloped by HARTIG's network, usually the nuclei lose their nucleoli and the nuclear structure becomes obscure. Granular bodies of tannic substances which are light yellowish green or green in colour are found dispersed in the cell-cavities in the outer cortex. Hyaline granules which have been described by MELIN, are found in the inner cortical cells. These bodies are not considered to be characteristics of mycorrhizas, the same bodies being found in the uninfected roots. Sometimes the fungous filaments enter into the cell-cavities forming haustoria.

In my opinion this is an ectotrophic mycorrhiza transformed by only one mycorrhizal fungus, no other kind of fungus being found in the mycorrhiza.

Form B.

This mycorrhiza is 0·32–0·45 mm. in thickness and also sends out branchlets dichotomously. The chief difference from Form A is that (1) the colour is pure white and (2) it has numerous projecting hyphae which bind neighbouring mycorrhizas up into a mass.

The fungous mantle is 18–23 μ in thickness, made up of rather loosely interwoven filaments, 1·5 μ in diam., with clump-connections. The depressed cells with much tannic substance are seen imbedded within the mantle. Beneath the mantle, there are usually four rows of cortical cells. The cells of the first row or pseudoepidermis are also filled up with masses of tannins. These cells are not only enveloped with HARTIG's network but also occasionally have intracellular filaments or haustorial hyphae which are 1·5–3 μ in diameter. They are not digested away by the host cells.

The second row of the cortex is made up of cells with minute granular substances or cells with granules measuring 1·3–3·1 μ in diameter. This layer corresponds to the "Körnerschicht" described by MELIN. He is of the following opinion: "Die erwähnten Körner in der Körnerschichte sind wahrscheinlich als eine Art von Exkretprodukten des Pilzes zu betrachten." These bodies are, however, not confined to the mycorrhiza,

the same ones being also found in the corresponding portion of the uninfected roots. Each cell of the layer is also enveloped by HARTIG's network and moreover has intracellularly infected hyphae which are similar in thickness to the haustorial hyphae but are ultimately cut up into pieces.

The third row of the cortex is made up of large cells also enveloped by HARTIG's network. They have granular tannins or hyaline bodies and also occasionally intracellular hyphae like the cells of the second raw.

The fourth row is the endodermis which is provided with much tannic substance.

According to MELIN's description, this mycorrhiza resembles bifurcated mycorrhiza type II and tubercle mycorrhiza, though there is no distinct digesting layer in the root-tissue.

It is quite clear that this is a mycorrhiza caused by one kind of fungus, no other kind of endophyte being found in the root-tissue.

Roots of *Pinus densiflora* and *Pinus silvestris* are much modified in their structure by the fungous infection, due perhaps chiefly to the chemical stimulation and also to the mechanical obstruction caused by the fungous mantle to the cells of their growing points. From the morphological investigation I have obtained no evidence from the above mentioned mycorrhizas that they represent symbiotic associations of fungi on roots.

II. SYNTHETIC INVESTIGATION.

The synthetic investigation was undertaken in order to ascertain if the above mentioned fungi are really mycorrhiza-formers.

A. Mycorrhizal Fungi in Pure Culture.

The materials for inoculation were taken (1) from the tissue of sporophores and (2) directly from the mycorrhizas. In all cases the cultures were started as soon as the specimens were brought into the laboratory.

The young sporophores or buttons were washed several times in sterile distilled water and were handled with sterile forceps. Another pair of forceps was used to remove the exterior portions from them, and the interior portions were cut into pieces with a sterile slender scalpel. The bits were then carefully inoculated on agar or gelatine plates. The inoculum was in most cases a centimeter or so in length. In the course of a month or so, the growing mycelium appeared on the culture medium.

The mycorrhizas were treated according to MELIN's method. The fresh materials were washed several times in sterile distilled water and treated for 15–30 seconds with a solution of corrosive sublimate (1 : 1000). They were washed again in sterile distilled water and then placed in the medium.

At first many media were used for these initial cultures. Later MEYER's (1) nutrient agar with glucose and (2) nutrient gelatine with glucose, (3) meat-extract-malt-extract agar and (4) malt-extract gelatine, with the following formulae, were used almost exclusively.

(1)	6 g.	peptone,
	4 g.	meat-extract (LIEBIG),
	1 g.	NaCl,
	500 cc.	water,
	5 g.	glucose,
	8 g.	agar,
(2)	500 cc.	water,
	6 g.	peptone,
	4 g.	meat-extract,
	1 g.	NaCl,
	5 g.	glucose,
	50 g.	gelatine,
(3)	2 g.	meat-extract,
	5 g.	malt-extract,
	100 cc.	MN + N (MEYER),[1]

1) 1 g. H_2KPO_4 + 0·1 g. NaCl + 0·1 g. $CaCl_2$ + 0·3 g. $MgSO_4 \cdot 7\ H_2O$ + 0·5 g. NH_4Cl + 0·01 g. Fe_2Cl_6 + 1000 cc. distilled water.

1·8 g. agar,
(4) 100 cc. MN+N (MEYER[1]),
5 g. malt-extract,
10 g. gelatine,

After the mycelium had grown around the inocula, small pieces of the growing mycelium were transferred from the petri-dish culture to ERLENMEYER-flasks or test tubes, containing nutritive medium (3), (4) or others.

Through these procedures, I could cultivate *Armillaria caligata*, *A. Matsudake*, *Boletus bovinus*, *Scleroderma vulgare* (?) and *Tricholoma Shimeji* from the sporophores, and *Boletus luteus* (?) from the tubercle mycorrhiza Form B of *Quercus pausidentata*, on the artificial medium. But *Cantharellus floccosus*, *Cortinarius cinnamomeus*, *C. sp. (a)*, *C. sp. (p)*, *Hydnum affine* and *Polyporus leucomelas*, in spite of repeated efforts, did not grow on the artificial medium.

1. *Armillaria caligata in culture.*

The mycelium of *Armillaria caligata* in pure culture grows rather deep in the artificial medium, always showing scanty white flying-mycelium (Pl. XI, Fig. 1, B). It grows very slowly on the usual artificial medium; it attains only a few centimaters in diameter in half a year.

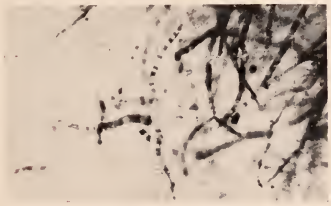

Fig. 50. Mycelium of *Armillaria caligata* in old stage in pure culture. ×420

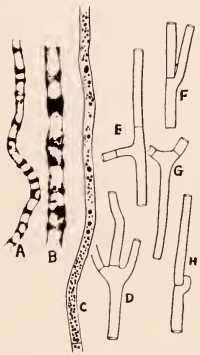

Fig. 51. Hyphae of *Armillaria caligata* in pure culture. *A* and *B*, old hyphae; *C*, young one; *H*, filament with clump-connection. ×780.

The mycelium gives a brown colour to the medium especially to the starch-agar medium (MN+N+1 % soluble starch+1·8 % agar) (Pl. XI, Fig. 1, A).

The mycelial filaments are hyaline, 1·2—4·7 μ in diam., and consist of minutely granulated very long cells usually with transverse septum and rarely with clump- connections (Text-fig. 51, *D–H*). The flying hyphae are much thinner than the hyphae found in the medium (Pl. XI, Fig. 3). When they become old, the cell-walls granulate or unevenly thicken as shown in the figure (Text-figs. 50 and 51, *A*, *B*). They do not produce spores on the artificial medium.

2. *Armillaria Matsudake in culture.*

The mycelium of this fungus, in pure culture, grows tolerably well on the artificial medium, producing cotton-like flying-mycelium (Pl. XI, Fig. 6).

Fig. 52. Mycelium of *Armillaria Matsudake* in pure culture. × ca 380.

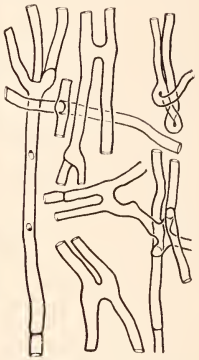

Fig. 53. Hyphae of *Armillaria Matsudake*. ×650.

The hyphae, made up of minutely granulated long hyphal cells, are hyaline, usually 1·5–3·1 μ sometimes 4·6–5·4 μ in diameter (Text-fig. 53). They show a tendency to coalesce with one another forming a bundle (Text-fig. 52). They do not produce spores on the artificial medium.

3. *Boletus bovinus in culture.*

The mycelium of this fungus in pure culture is white with strains of light yellow when fresh and young, changing to light yellowish brown with age, and gives a dark colour to the medium (Pl. XI, Fig. 2).

Fig. 54. Mycelium of *Boletus bovinus* in pure culture. ×500.

Fig. 55. Old hyphae projected from the natural mycorrhiza, showing locally swollen cell-wall. ×780.

Fig. 56. Old hyphae of *Boletus bovinus* in pure culture, showing locally swollen wall. ×780.

The hyphal filaments branch variously and are sometimes provided with clump-connections. They are 1·9–4·7 μ in diam., and frequently swell at various portions, attaining to 4·9–7·8 μ in diameter (Text-fig. 54, *S*). The young ones are composed of minutely granulated hyphal cells with even cell-walls, while, with age, the cell-wall thickens locally as found in the natural tubercle-mycorrhizas (Text-figs. 55 and 56 and Pl. XI, Fig. 8). They do not produce spores on the artificial medium.

4. *Boletus luteus* (?)[1] *in culture.*

The fungus was isolated from the compound mycorrhiza Form B of *Quercus pausidentata* (Masui, 1926, 3). The mycelium grows well on malt-extract-meat-extract agar, forming concentric rings of flying-mycelium

1) Dr. KRIEGER has kindly informed me that this fungus may be *Boletus granulatus* L.

Fig. 57. Mycelium of *Boletus luteus* (?) in pure culture. ×ca 660.

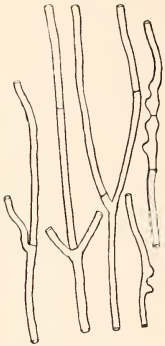

Fig. 58. Young hyphae of *Boletus luteus* (?) in pure culture. ×692.

(Pl. XI, Fig. 5).

The colour of the mycelium is light yellowish brown. The hyphae are 1·5–3·1 μ in diam., sometimes swelling on the artificial medium. They have usually transverse septum, and are lacking in true clump-connections. Frequently two branches are given off from one portion. When young,

Fig. 59. Old hyphae of *Boletus luteus* (?) in pure culture. ×692.

Fig. 60. Mycelium of *Tricholoma Shimeji* in pure culture. ×ca 460.

the hyphal cells have an even cell-wall, changing into a granular wall with age (Text-figs. 58 and 59).

5. *Scleroderma vulgare* (?) *in culture.*

The mycelium of this fungus grows very slowly on the artificial medium. Its colour is white with strains of light yellow. The hyphal characteristics are the same as those in the natural mycorrhizas.

6. *Tricholoma Shimeji in culture.*

The mycelium grows slowly on the artificial medium, forming radiate wrinkles (Pl. XI, Fig. 4). It is almost entirely without the flying mycelium. The colour of the mycelium is yellowish. The filaments, composed of very long hyphal cells with transverse septum, are usually 1·2–1·9 μ sometimes 4·6 μ diam., and rarely give off side-branches (Text- fig. 61). They coalesce with one another forming a bundle (Text-fig. 60). Hyphal anastomoses occur occasionally.

Fig. 61. Hypha of *Tricholoma Shimeji* in pure culture. ×692.

B. Sterilization of Seeds.

At first I employed several methods for the sterilization of seeds, but found following procedures to be excellent:

1. *Seeds of Pine.* In *Pinus densiflora* and *P. Tumbergii*, seeds of the previous year ripen during October in the vicinity of Kyoto. At this period the cones are still green with fast adhering scales, so that the preserved seeds are completely protected from the air. The seeds are already brown in colour and are capable of germinating.

Cones collected during October and September, were washed in 50% solution of alcohol and were transferred into a solution of corrosive sublimate (1 : 1000). In the solution, seeds were taken out from the cone

with a pair of sterile forceps. The seeds were washed in sterile distilled water and were then transferred into sterile Erlenmeyer-flasks for preservation or directly to agar medium in test tubes for immediate use.

Through this procedure, I could obtain almost 100 % of sterilized seeds with full capacity of germination.

Fig. 62. Seedlings of *Pinus densiflora* germinated from sterilized seeds. × 1·3

2. *Seeds of oaks.* The seeds of *Quercus* were washed in 50 % solution of alcohol for 10—20 minutes and were transferred into a solution of corrosive sublimate (1 : 1000). A pair of sterile forceps was used to remove the seed coat. Then they were thoroughly washed in sterile distilled water and transferred into sterile Erlenmeyer-flasks for preservation or directly to agar medium in large test tubes for immediate use.

The seeds sown on the media were incubated at 20°—25° for two weeks, and healthy seedlings were obtained.

C. Methods of Synthesis.

I used 500 cc.-Erlenmeyer-flasks for the pure culture of both fungi and vascular plants. As substratum (1) sand and (2) a nutrient solution or (3) a mixture of sand, humus and *Sphagnum*-moss and (4) another nutrient solution were prepared :

(1) The sand, whose granules were from 1 mm. – 3 mm. in diam., was used after washing first with mineral acid and then thoroughly in water.

(2) The nutrient solution used [(Meyer's MN + N) + glucose] has the following formula :

1000 cc.	Distilled water,
1 g.	KH_2PO_4,
0·1 g.	$CaCl_2$,
0·1 g.	NaCl,
0·3 g.	$MgSO_4 . 7H_2O$,
0·01 g.	Fe_2Cl_6,
0·5 g.	NH_4Cl,
0·5 g.	glucose.

(3) The sand, humus and *Sphagnum*-moss were mixed in the following proportion:

sand (granules, 1 mm. – 3 mm. in diam.) 3 vol.,
humus (particles, 1 mm. – 10 mm. in diam.) 3 vol.,
Sphagnum-moss (particles, 1 mm. – 5 mm. in diam.) 1 vol.

(4) The nutrient solution, used with (3), has the following formula:

1000 cc.	tap water,
0·5 g.	glucose.

I put 150 cc. of the sand (1) and 37 cc. of the first nutrient solution (2), or the same volumes of the mixture (3) and the second solution (4) in each flask, and autoclaved for 30 minutes at 150°—160°.

The flasks with both the fungus and the seedlings were placed in a green house.

D. The Results of the Synthesis.

1. *Armillaria caligata* + *Pinus densiflora*.

For the synthesis between *Armillaria caligata* and *Pinus densiflora*, I used at first large test-tubes, 3 cm. in diam., Knop-solution and glass-cotton as the substratum. After they had been autoclaved, the sterilized seeds of *Pinus densiflora* were sown. They were germinated in the tubes with quite a normal aspect. Small blocks of the fresh mycelium of *Armillaria caligata* in pure culture were then put in the tubes and buried near the roots of the seedlings. In two months, the mycelium showed rapid growth along the roots and at last killed them, while those

in control grew normally. This evidence indicates that the fungus, in this case, is parasitic on *Pinus densiflora*.

On the 29th of October, 1924, I planted sterilized seedlings in the sand with MN+N+glucose in the ERLENMEYER-flasks. The seedlings were grown normally within them. On the 20th of April, 1925, I put inocula into the flasks and buried them near the seedlings. After three months the mycelium had made enormous development covering the roots with a pure white mycelial network (Text-fig. 63). The infected roots were limited to those found in the superficial layer of the medin, those in the deeper layer being almost free from the mycelium.

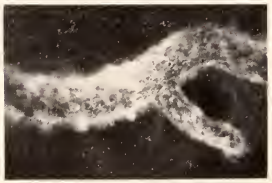

Fig. 63. The effect of the synthesis. A root of *Pinus densiflora* enveloped by the mycelium of *Armillaria caligata*. × 16.

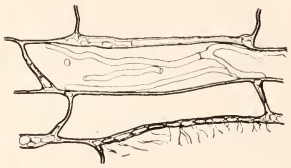

Fig. 64. The effect of the synthesis. Longitudinal section of a mycorrhizal root, showing HARTIG's network and intracellular filaments. × 578.

The microtonic section of the infected roots revealed a well-developed HARTIG's network, made up of filaments, 1·5–2 μ in diam., and intracellular hyphae, 2·5—3·2 μ in diameter (Text-fig, 64) as shown in the natual mycorrhiza (page 157). The fungous mantle was however rather rough in texture contrary to that of the natural ones.

It was thus clearly shown synthetically that *Armillaria caligata* can be one of the mycorrhizal fungi of *Pinus densiflora*.

2. *Armillaria Matsudake* + *Pinus densiflora*.

When both the inocula and seedlings are simultaneously transferred into the substratum in the flasks, the young roots of the former are so seriously infected that they are ultimately killed (Text-fig. 65). I therefore

added the inoculum, when the seedling had attained several centimeters. The most successful results were obtained in a set of cultures in which the seedlings were sown on the 18th of October, 1923, and the inocula

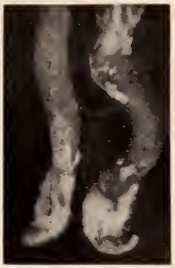

Fig. 65. Heavily infected roots of seedlings.

Fig. 66. Pure culture of the mycelium of *A. Matsudake* and *Pinus densiflora*. × ca 0.5

Fig. 67. A pine, shown in Text-fig. 66, showing heavily infected roots. × ca 1.3

on the 15th of January of the year. Two months after that, the seedlings entirely ceased further growth, turning yellowish in colour (Text-fig. 66), while those of the control grew normally maintaining a vivid green colour. On the 25th of May, the plants in the experiment were examined. I found then that all the roots, had been enveloped by a thick mycelium as shown in the figure (Text-fig. 64 and Pl. XI, Fig. 9). The microtomic section of the roots showed well-developed intercellular and intracellular hyphae. The filaments diverged intercellularly entering into the neighbouring

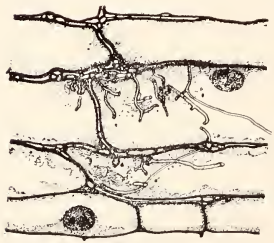

Fig. 68. Longitudinal section of a mycorrhiza of *Pinus densiflora* caused by *A. Matsudake* in the synthesis. × ca 400.

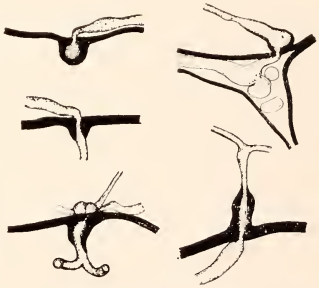

Fig. 69. Fungous filaments entering into the cell-cavity dissolving the cell-wall, in the synthetic culture. × 1040.

cells dissolving the cell-wall, and moreover the intracellular filaments entered directly into the other cells (Text-fig. 68). When the filaments enter into the cell cavity, they are enveloped by thick membranaceous substances (Text-fig. 69). These facts agree with those in natural mycorrhizas.

The fungous mantle is, though thick, rather rough in texture.

The experiment shows clearly that *Armillaria Matsudake* is also one of the mycorrhizal fungi of *Pinus densiflora*.

3. *Boletus bovinus* + *Pinus densiflora*.

Four months after the germination of the seeds in large test-tubes,

blocks of fresh mycelium of the fungus in pure culture were put into them. The seedlings in the investigation were seriously enfeebled and at last killed by the infecting mycelium within a month, while those of the control grew normally. The roots were abnormally thickened at the infected portion, giving off almost no side-branches. This portion, was dark brown in colour.

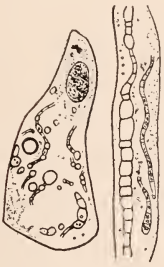

Fig. 70. Intracellularly infected hyphae of *Boletus bovinus*. × 520.

The microtomic section of such roots revealed enormously developed intracellular hyphae in the cortical tissue. They are septated filaments varying from 1·6–7·7 μ in diameter (Text-fig. 70).

On the 4th of September, 1925, the seedlings germinated from the sterilized seeds were transferred to a substratum, (1) + (2), in the flasks. The seedlings grew normally within them. On the 12th of October, 1925, small blocks of the mycelium of this fungus were put into the flasks and placed in the green house. After two months mycelium was developed encircling the seedlings. Moreover even the lower portion of the stems was enveloped by the white mycelium, as shown in the figure (Pl. XI, Fig. 7, *M*). On examining the seedlings in the flasks on the 6th of January of the following year. I

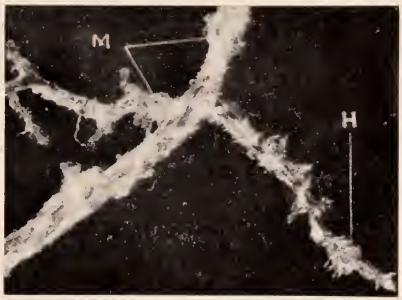

Fig. 71. Seriously infected root of *Pinus densiflora*. *H*, root hairs; *M*, mycelium. × 12.

found that not only had the mycelium grown luxuriantly on the roots forming typical mycorrhizas, but also that the fungous filaments given rise to from their surfaces had cemented the surrounding soil into a mass. These filaments were proved by microscopical investigation to be those of *B. bovinus*. The infected portion of the root had no root-hairs, while uninfected portions and roots lying deeper in the flask had them (Text-figs. 71 and 72).

Fig. 72. Mycorrhizas of *P. densiflora* caused by *Boletus bovinus* in the synthesis. × 25.

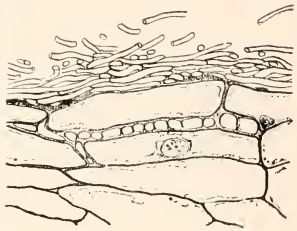

Fig. 73. Longitudinal section of a mycorrhiza of *P. densiflora* caused by *Boletus bovinus* in the synthetic culture. × 578.

The microtomic sections stained with DELAFIELD's haematoxylin showed that the rootlets had been actually transformed into mycorrhizas, that is to say, they had not only a fully-developed fungous mantle, measuring 10—54 μ in thickness, but also HARTIG's network (Text-fig. 73). I found in older mycorrhizas that they had exactly depressed cortical cells, bearing the fungous filaments inserted between them. The thick fungous mantles which had nearly perished, were always found bordered with such cortical tissue in sections. The younger mycorrhizas, however, had normal cortical tissue which had the filaments inserted between the cells. They were large filaments measuring 2—4·6 μ in thickness. The fungous mantle was made up of interwoven filaments measuring 1·5—2 μ in diameter. I could not find any filaments that had entered the

cell-cavity of the host tissue.

4. *Boletus luteus (?) (mycelium isolated from compound mycorrhiza Form B of Quercus pausidentata) + Quercus myrsinaefolia.*

I used the mixture of sand, humus and *Sphagnum*-moss (3) and the nutrient solution (4) as a substratum. The seedlings, germinated

Fig. 74. Mycorrhizas of *Quercus myrsinaefolia* caused by *Boletus luteus* (?). ×6.5.

from sterilized seeds, and small blocks of the fresh mycelium of this fungus were transferred simultaneously to the substratum in flasks. During two months or so, the seedlings attained a length of several centimeters and the mycelium diverged all over the substratum. After three months and a half, the plants in the experiment were examined. Observation with a low-powered microscope revealed that the growth of the roots, especially that of the rootlets, had been seriously inhibited by the mycelial infection. They were brown or dark brown in colour and entirely devoid of root hairs (Text-fig. 74).

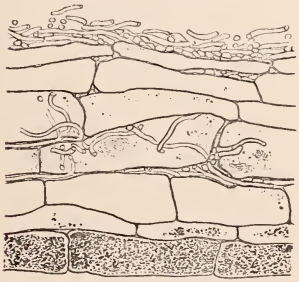

Fig. 75. Longitudinal section of a mycorrhiza of *Q. myrsinaefolia* caused by *Boletus luteus (?)* in synthesis. E, endodermis. ×578.

Microtomic sections of them showed a rather thin fungous mantle, 8—15 μ in width, interwoven by filaments measuring 1·5—2·3 μ in diameter. There were several rows of cortical cells. The intercellular filaments had entered into the cell-cavity dissolving the cell-wall, forming endotrophic filaments. They were also 1·5—2·3 μ in diameter. The endodermal cells contained a large amount of tannic substances as shown in the figure (Text-fig. 75).

5. *Boletus luteus* (?) + *Pinus Tumbergii*.

As a substratum, (3)+(4) was used. The seedlings and the inocula were transferred to the flasks simultaneously on the 28th of December, 1925. The flasks were placed in a green house. The seedlings in the experiment were not so healthy as those of the control. The fungous filaments grew luxuriantly on the young roots transforming them into typical mycorrhizas (Pl. XI, Fig. 10). The roots entirely lacked root-hairs on their surfaces.

Microtomic sections of them showed a well developed intercellular

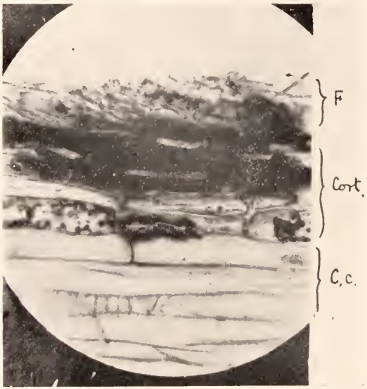

Fig. 76. Longitudinal section of a mycorrhiza of *P. Tumbergii* caused by *B. luteus* (?). *F*, fungous mantle; *Cort*, cortex *C.c.*, central cylinder.

mycelium, made up of granulated filaments measuring 1·5—2·3 μ in diam., and intracellular filaments of the same thickness. The fungous mantle is rather thin, 8—16 μ in width, and rough in texture (Text-figs. 76 and 77).

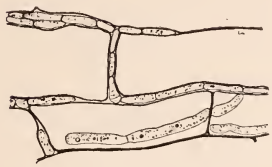

Fig 77. Cortical cells of the mycorrhiza, showing intercellular and intracellular filaments. ×780.

6. *Boletus luteus* (?) + *Quercus phylliraeoides*.

The substratum used was (3)+(4). The inoculum and the seedling were transferred simultaneously to it in the flask. In two months the roots were seriously infected by the

Fig. 78. Mycorrhizas of *Quercus phylliraeoides* caused by *Boletus luteus* (?). ×6.

mycelium until they grew no more, turning brown or dark brown. The fatally invaded roots were enveloped by a white mycelium (Text-fig. 78).

Microtomic section of the heavily infected roots showed that all the cells of the cortical tissue had been depressed into one thin layer. The fungous filaments, 1·5—2·3. μ in diam., were found inserted among the depressed cells and around the cortex forming a roughly interwoven mantle.

7. *Boletus luteus (?) + Quercus glauca.*

The substratum used was (3)+(4). The inoculum and the seedling were simultaneously transferred to it in the flask.

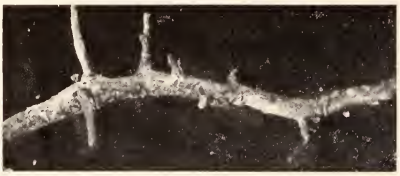

Fig. 79. Mycorrhizas of *Quercus glauca* caused by *Boletus luteus* (?). ×6.

Within two months, the roots had been seriously invaded by the mycelium turning dark brown in colour (Text-fig. 79). The younger mycorrhizas had a faint fungous mantle, intercellular and intracellular filaments (Text-fig. 80), while in older ones, the roots being killed, they had depressed cortical tissue.

Fig. 80. Longitudinal section of a mycorrhiza of *Quercus glauca* caused by *Boletus luteus* (?). ×578.

8. *Boletus luteus (?) + Quercus grosseserrata.*

The substratum used was (3)+(4). The inoculum and the seedling

were simultaneously transferred to it in the flask.

The fungous filaments grew densely on the surface of the roots transforming them into mycorrhizas (Text-fig. 81). They had the fungous mantle, the intercellular and intracellular filaments.

Fig. 81. Mycorrhizas of *Quercus grosseserrata* caused by *Boletus luteus* (?). ×6.

9. *Boletus luteus (?) + Quercus pausidentata.*

The fungus grew luxuriantly on the roots transforming them into single mycorrhizas. But in the experiment, in vitro, no tubercle-mycorrhizas were formed.

10. *Tricholoma Shimeji + Pinus densiflora.*

On the 5th of October, 1925, I sowed sterilized seeds of *Pinus densiflora* in a soil (3)+(4), and at the same time transferred small blocks of the mycelium of *Tricholoma Shimeji*, in pure culture, also to the soil in a Erlenmeyer-flask. The seeds germinated in 15—17 days in the green house. The plants, on the one hand, grew normally, extending their roots along the basal surface of the flask. On the other hand, white mycelium radially diverged from the inoculum, maintaining its characteristic manner of growing. The mycelium began to grow luxuriantly in spring around the young side-branches until they were transformed into mycorrhizas, while the main root maintained its further growth. These phenomena were successively observed with a pocket lens from the

underside of the flask.

The plants, in the experiment, were examined on the 1st of July, 1926. The plants had light green, delicate leaves and very long main roots. The branches, given off from the root, were very short clavate or dichotomously branched mycorrhizas (Text-fig. 82).

Fig. 82. The effect of the synthetic investigation, showing mycorrhizas of *Pinus densiflora* caused by *Tricholoma Shimeji*. ×3.5.

Microtomic sections proved them to be normal mycorrhizas. They were composed of a well-developed central cylinder, rather thin cortical layer and the fungous mantle. The fungous mantle was usually very thin, sometimes almost lacking. The cortical tissue had a typically developed HARTIG's network (Text-fig. 83). The intercellular hyphae, 1·5—4·0 μ in diam., entered into the cell-cavity dissolving the cell-wall. Where the hyphae had just begun to enter the cell-cavity, thick membraneous sub-

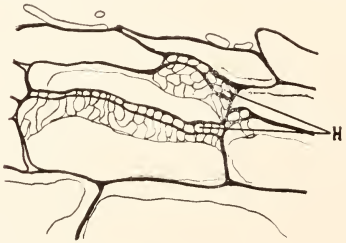

Fig. 83. Longitudinal section of a mycorrhiza of *Pinus densiflora* caused by *Tricholoma Shimeji*, showing well-developed HARTIG's network, *H*. ×578.

Fig. 84. A cortical cell of a mycorrhiza of *Pinus densiflora* caused by *Tricholoma Shimeji*, showing intracellular filaments, *a*, and intercellular ones on the point of entering the cell-cavity, *b*, dissolving the cell wall. ×578.

stances were usually formed at that portion around the heads of the hyphae. (Text–fig. 84).

E. General Results of the Synthesis.

1. The synthetic investigation proved the following fungi to be mycorrhiza formers on vascular plants:

Armillaria caligata on *Pinus densiflora*,
A. Matsudake on do.,
Boletus bovinus on do.,
Tricholoma Shimeji on do.,
Boletus luteus (?) on *Pinus Tumbergii*, *Quercus myrsinaefolia*, *Q. phylliraeoides*, *Q. glauca*, *Q. grosseserrata* and *Q. psusidentata*.

2. The structure of the mycorrhizas, formed in vitro, differs more or less from natural ones. The chief difference to be mentioned is as follows: a. The fungous mantle in the culture is usually thin and rough in texture, and sometimes lacking; while in natural ones the mantle is usually thick and dense in texture. b. As the intracellular mycelium develops well in synthetic culture, the infected roots are killed sooner than in the natural case.

III. SEASONAL RELATION OF MYCORRHIZAS.

I have observed the mycorrhizas for four years, finding that each kind of mycorrhiza has more or less a different period of prosperity.

In the case of the mycorrhiza Forms A and B of *Alnus japonica*,

well-developed fresh mycorrhizas begin to increase in number from April to May, attaining their maximum during June, July and August (Masui, 4). Throughout the months of November to February good fresh specimens are rather scarce, while old, dead ones are very plentiful.

The mycorrhizas of *Populus tremula v. villosa* caused by *Cortinarius cinnamomeus* are plentiful during April and May. This condition persists till autumn.

All kinds of the mycorrhiza of *Pinus densiflora* studied have almost the same seasonal fluctuation. The fresh mycorrhizas increase in number during May and June. By the middle of summer, however, the mycorrhizas begin to die off, and good specimens become rather scarce. During September and October, the fresh mycorrhizas suddenly increase again attaining their maximum number. They then decrease toward December, and the decline persists from January to April.

A most interesting fact is that the fresh mycorrhizas of *Pinus densiflora* caused by *Cortinarius cinnamomeus* are very scarce during April and May, while fresh mycorrhizas of *Populus tremula v. villosa* caused by the same fungus are plentiful during these periods.

These facts indicate undoubtedly that the seasonal variation of the mycorrhiza is intimately related with the growth period of the host root (compare further p. 259).

IV. MICROCHEMICAL INVESTIGATIONS INTO THE NORMAL AND MYCORRHIZAL ROOTS AND FRUITING BODIES OF THE MYCORRHIZAL FUNGI.

In order to make clear the nutritive relation between the root of the mycorrhiza-producing plants and the mycelium of the mycorrhizal fungi, I made microchemical tests upon both infected and uninfected roots, as has been already done by WEYLAND (1912), REXHAUSEN (1920) and MASUI (1926), and moreover upon young fruiting bodies of mycorrhizal fungi. The materials used were as follows :

1. Normal roots and mycorrhizas Form A of *Alnus japonica* (Masui, 4);
2. Normal roots and *Boletus bovinus*-mycorrhizas of *Pinus densiflora*;
3. Normal roots and *Hydnum affine*-mycorrhizas of *Pinus densiflora*;
4. Normal roots and mycorrhizas Form B of *Abies firma* (Masui, 1);
5. Normal roots and compound mycorrhizas Form A of *Quercus pausidentata* (Masui, 3);
6. Normal roots and mycorrhizas of *Pinus silvestris*;
7. Normal roots and cluster-type mycorrhizas of *Pinus densiflora* cultivated in sand and humous soil respectively;
8. Buttons of *Armillaria Matsudake*, *Boletus bovinus* and *Cortinarius sp. (a)*.

A. Methods Employed.

Microchemical methods employed were as follow:

1. *Amino-acids.* For the test of amino-acids in material, I used 1 % aqueous solution of ninhydrin introduced by O. Loew (1917). According to his description, 1 % ninhydrin solution reacts, in room temperature, upon alanine, leucine and histidine in 15 minutes, lysine and arginine in ca 20 minutes, asparagine-acid and glutamine-acid in ca 2 hours, phenylalanine in 3 hours in all cases giving colouring reactions. Asparagine, being an amide of an asparagine-acid, give a reddish yellow colour, changing into deep red brown on heating.
2. *Albuminous substance.* For the detection of albuminous substance in mycorrhizas, sometimes Millon's reagent was unfavourable, as the reaction colour is confused with the brown calyptral layer which is always found beneath the fungous mantle. The xantho-protein reaction, biuret reaction and Raspail's reaction proved

to be very favourable for this purpose.

3. *Sugar.* For the detection of sugar in the material, MEYER's method and *α*-naphtole–sulphuric acid were used with good results.

4. *Glycogen.* An iodine–potassium iodide solution and sometimes FISCHER's tannin–safranine–staining method were used.

5. *Starch.* The usual iodine–potassium iodide solution.

6. *Mucilagenous substances.* An aqueous solution of ruthenium–red.

7. *Tannic substance.* I used an aqueous solution of iron sulphate as well as a mixed solution of ammonium molybdate and ammonium chloride. The former gives a deep blue colour, and the latter a reddish brown colour.

8. *Phosphorus.* For the detection of phosphorus in the material I used fresh FRESENIUS' solution and 2 % solution of phenylhydrazine chloride which has been used by WEVLAND and REXHAUSEN. The sections of the material dipped in excess of the first solution for 20 minutes or more in the room temperature, 15—20°C, washed for 6—12 hours in acidulated distilled water (100 cc. water with several drops of concentrated solution of nitric acid). For the reduction of phospho-ammonium-molybdate I used at first a 20 % solution of pyrogallol. But I found that the solution was not suitable for these materials as the colour given by it is much confused with the brown calyptral cells and tannic substance. 2 % solution of phenylhydrazine chloride, which gives from a green to a blue colour, was found to be a superior reagent for the reduction. Tannic substances which are found in large amounts in the root tissue turn to a dark colour by treatment with these reagents.

9. *Nitrate.* For the detection of nitrates in the material I used diphenylamine–sulphuric acid introduced by MOLISCH which gives a deep blue colour to them.

10. *Potassium* (and ammonium). For the detection of potassium

and ammonium together in the material I employed Molisch's sodium-cobalt nitrite method with excellent results.

11. *Ammonium.* I could not find a suitable reagent for the detection of ammonium, so that I was compelled to use Nessler's reagent which gives a yellowish brown precipitation to ammonium.

B. The Results of the Investigation.

1. *Normal roots and mycorrhizas Form A of Alnus japonica.*

(1) *Morphological difference between the normal and the mycorrhizal root.*

In order to make clear the nutritive relation between the root and the mycelium it is necessary, in the first place, to know the morphological features of both the normal and mycorrhizal roots.

The mycorrhiza Form A of *Alnus japonica* differs very much from the normal root. In external characteristics, the former is a delicate, irregulary bent, while the latter is a stout, ustually straight, white or yellowish one.

The length of the mycorrhiza is usually 5—11 mm., sometimes attaining 18 mm. In long ones, the apical portion often retains a white colour, though the basal portion becomes brownish little by little and ultimately perishes. Cross section of the half decayed portion revealed the cells filled up with mucilagenous substances, indicating that they have lost the vitality. From the external appearance as well as from the internal structure, it may be easily concluded that the mycorrhizas become decrepit gradually from their basal portion.

The mycelium projected from the surface of the white portion is also white and vigorous while that found around the brownish basal portion appears almost decayed.

Cross section of the normal roots, made successively from the apical point backwards, shows that (1) the diameter of the central cylinder, (2) the number and size of tracheids and (3) the

area of tracheidal bundles increase gradually toward the basal portion (Tables 1—3 and Text-figs. 85 and 86).

Successive cross sections of the mycorrhizal roots, made in the same

TABLE 1.

Normal root.....(A)

Distance from the tip(mm).	*Diam. of the root (mm).*	*Diam. of the cent. cylinder (mm).*	*Number of tracheids.*	*Size of tracheids (long diam.).*
3	0·62	0·23	20	7·1—10·2
10	0·93	0·28	45	7·1—14·2
20	0·94	0·28	90	14·3—21·4
30	0·89	0·36	216	14·3—35·7
40	0·80	0·45	529	14·3—42·8

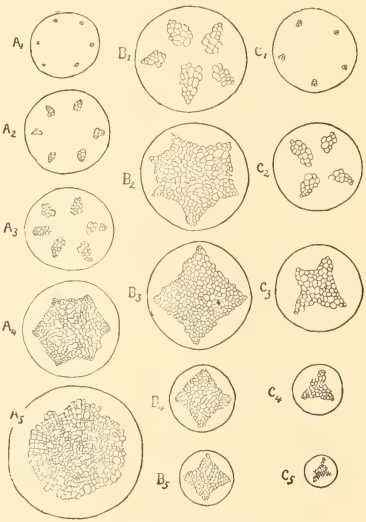

Fig. 85. The development of tracheids in a normal root, *A1–A5*, and mycorrhizal roots, *B1–B5* and *C1–C5*, of *Alnus japonica*.

way, show just opposite results in these three points i. s. the diameter of the central cylinder in the basal portion is usually ½—⅓ of that of the apical portion, and moreover

TABLE 2.

Mycorrhizal root.....(B)

Distance from the tip (mm).	*Diam. of the root (mm.).*	*Diam. of the cent. cylinder.*	*Number of tracheids.*	*Size of tracheids (long diam.).*
2	0·62	0·36	85	14·3—50
3	0·62	0·39	272	14·3—50
4	0·62	0·39	302	8·5—32·2
6	0·55	0·26	151	7·1—21·4
8	0·53	0·16	118	7·1—8·8

the tracheids in the former are very small and fewer in number than those of the latter. These relations are quite constant in numerous mycorrhizas of various lengths, as if the substances in the

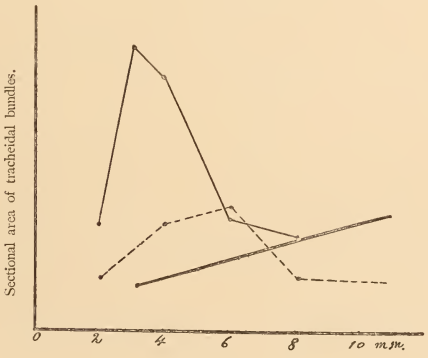

Fig. 86. The mode of development of tracheidal bundles of *Alnus japonica* according to the position of roots from the apex onwards in normal and mycorrhizal roots.
——, normal root, *(A)*;—, mycorrhizal root, *(B)*; ----, mycorrhizal root, *(C)*.

TABLE 3.

Mycorrhizal root.....(C)

Distance from the tip (mm).	*Diam. of the root (mm.).*	*Diam. of the cent. cylinder.*	*Number of tracheids.*	*Size of tracheids (long diam.).*
2	0·57	0·36	19	8·2—22
4	0·55	0·36	52	14·3—29
6	0·50	0·27	77	14·3—29
8	0·45	0·20	56	8·2—21·3
10	0·41	0·12	43	7·1—14

mycorrhizas are translocated forwards contrary to the normal root (Text-figs. 85 and 86).

(2) *Mucillagenous substances.*

Reagent ; ruthenium-red.

a. *Mycorrhiza* (Table 4).

TABLE 4.

	Colour given by the reagent.		
Portions of mycorrhizas examined.	*Apical portion.*	*Middle portion.*	*Basal portion.*
Fungous mantle.	*red*	*red*	*red*
Cortex.	*light red.*	*red — deep red*	*deep red.*
Central cylinder.	*light red.*	*red — deep red.*	*deep red.*

b. *Normal root.* Every portion of the normal root is almost devoid of the mucilagenous substances.

The most interesing thing about this mycorrhiza is that all the tracheids in the basal portion of it are filled up with mucilagenous substances, while in the normal root they are entirely empty.

The fungous mantle is given red colour by treatment with this reagent, indicating that it contains much glycogen.

(3) *Tannic substance.*

Reagents: (1) iron sulphate and (2) ammonium molybdate.

Tannic substances are found contained not only in the cell-wall, but also in the granules in the cell-cavities of the root cells.

a. *Tannic substances contained in cell-walls* (Table 5):

TABLE 5.

	Portions examined	*Apical portion.*	*Middle portion.*	*Basal portion.*
Normal root.	*Cortex.*	++		++
	Central cylinder.	++		+
Mycorrhizal root.	*Cortex*	++	++	(+)
	Central cylinder.	++	++	++

b. *Tannic substances contained in cell-cavities* (Table 6):

TABLE 6.

	Portions examined.	*Apical portion.*	*Middle portion.*	*Basal portion.*
Normal root	*Cortex*	++ *(root-cap)*		+
	Central cylinder			+
Mycorrhizal root.	*Cortex*	+	++	++
	Central cylinder	+	++	+++

As the tables show, the basal portion of the normal roots contains a slightly larger amount of tannic substance than the same portion of the mycorrhizal roots, while the latter has a far larger amount of the granular tannins in the cell-cavities than the former.

A most remarkable fact in this case is that all the tracheids in the basal portion of the mycorrhizal root are entirely filled up with dense tannins as shown in the figure (Text-fig. 87).

There are, in most cases, side branches whose apical portions have been transformed into mycorrhizas. Cross section of the such mycorrhizas usually shows the cells of both the central cylinder and

the endodermis filled up with tannic substances as in the basal portion of the normal mycorrhizas (Text-fig. 88).

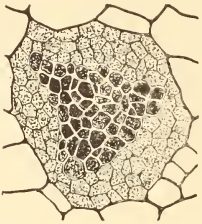

Fig. 87. Cross section of the basal portion of a mycorrhiza, showing tracheids filled up with tannic substances.

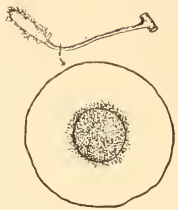

Fig. 88. Cross section of the stalk of a mycorrhiza, showing a large quantity of tannic substances (dots) in the central portion.

(4) *Amino-acids.*

Reagent : 1 % solution of ninhydrin.

The reagent gives a blue or violet colour to amino-acids.

At first the experiment was performed upon preparations of the whole mycorrhiza. The old mycorrhizas together with enveloping mycelium including humous particles were compared then with the young ones, which have fewer projecting filuments. On treatment with the reagent, the young mycorrhiza began to gain a bluish colour in 15 minutes, the colour gradually deepening to dark violet in 1 ½ hours, while the old one gained only a light violet colour. The middle-aged one gained a deep colour only at its termination.

The reagent gave a light violet colour to the hyphae projected from the mycorrhizal surface. The colour reaction of the mycelium found near the mycorrhiza was always deeper than that at a distance (Text-fig. 89. *C*). Moreover, the hyphal mass around the mycorrhiza, even if it had been exposed to the air, also gained a deep violet colour (Text-fig. 89, *A* and *B*). All these evidences seem to indicate that amino-acids in the mycelium are supplied from the root.

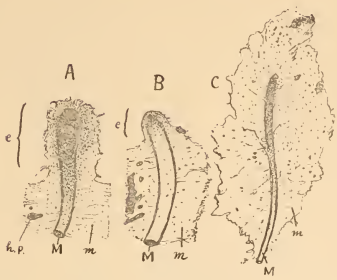

Fig. 89. Total mycorrhizas treated with ninhydrin. *A*, a mycorrhiza half exposed to the air. *e*, The mycelium of the exposed portion which gave deep violet. ×5. *B*, a rather old mycorrhiza which gave a rather deep colour only at its terminal end. The mycelium around the apex gave a violet colour exclusively. ×5. *C*, a mycorrhiza with large mass of flattened mycelium developed on a rotten leaf. The mycelium gave deeper colour only around the mycorrhiza. ×4. *M*, mycorrhiza; *m*, mycelium; *h. p.*, humous particle. Dots mean amino-acids.

a. Sections of a normal large root, 1·5 mm. in diam., and a normal side-branch, 8 mm. long and 0·5 mm. in diam., were examined and gave the following results (Table 7):

TABLE 7.

Distance from the apex in mm.	*Portions of roots examined.*	*Grades of colour given by the reagent, after 2 hours. 10 indicates maximum depth of the colour and 1 the minimum.*	
		Large normal root.	*Normal side-branch.*
1.	*Outer margin of the central cylinder.*		10.*(deep violet)*
	Cortex.		10.
3.	*Outer margin of the central cylinder.*	10.	8—10.
	Cortex.	6—7.*(violet)*	7—9.
5.	*Outer margin of the central cylinder.*		7—9.
	Cortex.		6—8.
7.	*Outer margin of the central cylinder.*		5—7.
	Cortex.		4—5.

Distance from the apex in mm.	*Portions of roots examined.*	*Grades of colour given by the reagent, after 2 hours. 10 indicates maximum depth of the colour and 1 the minimum.*	
		Large normal root.	*Normal side-branch.*
10.	*Outer margin of the central cylinder.*	6—7.	
	Cortex.	2.	
30.	*Outer margin of the central cylinder.*	5.	
	Cortex.	1—2.	
60.	*Outer margin of the central cylinder.*	3—4.	
	Cortex.	1.	

b, Mycorrhizas just completed, middle-aged ones and old ones with long projecting hyphae all over their surfaces were examined and gave the following result (Table 8);

TABLE 8.

Distance from the apex. mm.	*Portions examined.*	*Grades of colour given by the reagent after 2 h. 10 indicates maximum of the colour; 1, minimum.*		
		Very young mycorrhiza.	*Middle-aged mycorrhiza.*	*Old mycorrhiza.*
1.	*Fungous mantle.*	3—4.	2—3.	1—2.
	Outer margin of the central cylinder.	8—10.	4—5.	1—2.
	Cortex.	8—10.	3—5.	0—1.
3.	*Fungous mantle.*	2.	2.	1—2.
	Outer margin of the central cylinder.	5—6.	2—3.	1.
	Cortex.	4—5.	1—2.	0—1.
5.	*Fungous mantle.*	2.	2.	0—2.
	Outer margin of the central cylinder.	4—6.	1—3.	0—1.
	Cortex.	3—5.	0—2.	0—1.
7.	*Fungous mantle.*	2.	2.	1—2.
	Outer margin of the central cylinder.	4—6.	1—3.	0—1.
	Cortex.	3—5.	0—2.	0—1.

In order to detect asparagine in the root tissue, materials were boiled

in the reagent, but no yellowish colour was given, indicating that the material lacks this substance.

The uninfected growing root, without regard to its thickness, has a great quantity of amino-acids in the apical portion, as well as in the outer margin of the central cylinder. The colouring decreases basipetally, indicating that amino-acids are food-substances translocated to the growing point of the root from the mother root (Text-fig. 90, *A*).

Fig. 90. Localisation of amino-acids, in the normal and mycorrhizal roots, young and old, treated with 1 % solution of ninhydrin shown in longitudinal section. *A*, normal side-branch; *B*, very young, *C*, middle-aged and *D*, old mycorrhiza. *c*, cortex; *cc*, central cylinder; *f*, fungous mantle; *m*, projecting mycelium. Dots indicate amino-acids.

Very young mycorrhizas just completed have red pigment at the apical portion of the roots as in the case of normal roots, and almost lack the projecting hyphae at the apical end of them. Such young mycorrhizas have a great deal of amino-acids in the apical portion and in the outer margin of the central cylinder, though the amount is a little less than in the corresponding portions of the normal root (Text-fig. 90, *B*). The most novel thing about this stage of the mycorrhiza is that the fungous

mantle which envelops the root has a greater amount of amino-acids in its apical region than in the basal, though the former has no intimate connection with the humus outside, as it lacks the projecting hyphae.

The middle-aged mycorrhiza has a less amount of amino-acids in the root tissue than the young one, though it has long projecting hyphae all over its surface.

The old mycorrhiza has almost no amino-acids in the root-tissue, and only a small amount of them in the fungous mantle, though it has very long and very many hyphae in contact with the humous soil.

All these facts clearly show that amino-acids which had once been contained, in large amount, in the young mycorrhizal root have been used up by the infected fungus with age.

(5) *Albuminous substances.*

Method employed: (1) xanthoprotein reaction, (2) biuret reaction and (3) RASPAIL'S reaction.

These reactions equally showed a great deal of albuminous substances in the fungous mantle, a very small amount in the cortex and a small amount in the central cylinder of the mycorrhiza. The quantitative difference between the normal and mycorrhizal roots, in the cortex and the central cylinder, was almost undistinguishable.

(6) *Sugar.*

Method used for the detection of sugar: (1) MEYER'S method and (2) *α*-naphtole-sulphuric acid.

Longitudinal sections of both large and small normal roots, treated with the reagents, showed that uninfected roots have, without regard to their thickness, a great quantity of sugar at the growing point and a small amount in the basal parts (Table 9), indicating that sugar is a substance transported to the growing point.

Generally the mycorrhiza has less sugar than the normal root without regard to its portions (Table 9). In the young mycorrhiza, however, a rather large amount of sugar is found in the apical portion of the central cylinder, though, with age, it is gradually exausted.

TABLE 9.

	Portions examined.	Colour given by MEYER's method.	Colour given by α-naphtole-sulphuric acid.
Normal root. (Apical portion.)	Cortex.	Yellowish brown—reddish brown.	Very light purple—light purple.
	Central cylinder.	deep reddish brown.	light purple—purple.
Middle-aged mycorrhiza. (Apical portion).	Fungous mantle.	light yellow—yellowish brown.	deep violet.
	Cortex.	light yellowish brown.	very light purple—light purple.
	Central cylinder.	light yellowish brown—yellowish brown.	very light purple—light purple.

The reason why the mycelium turns deep violet when it is treated with α-naphtole and concentrated solution of sulphuric acid, may be that by the treatment with such a concentrated mineral acid, the glycogen is broken up into great quantity of hexoses.

In a word, the normal root has more sugar than the mycorrhizal root, indicating without doubt that the sugar is absorbed by the infected fungus.

(7) *Glycogen.*

When the sections of the mycorrhizas are treated with iodine-potassium iodide solutions, the fungous mantle is given a deep reddish brown colour, which fades on heating.

FISCHER's tannin-safranin-staining method proves also the existence of glycogen, in great quantity, in the fungous mantle.

It occurs in the mycorrhiza itself during its development, that the amount of sugar in the root tissue is gradually exausted, and on the other hand the glycogen is increased in the

fungous mantle. This fact made me concieve that a large amount of sugar which has been taken off from the root tissue may be transformed into glycogen in the fungous cells.

(8) *Starch.*

No starch grains are found in the tissues of the mycorrhiza.

The young normal root also lacks starch grains, though a large amount of it is found in its older portions.

(9) *Phosphorus.*

Reagent: FRESENIUS' solution and 2 % solution of phenylhydrazine chloride.

The results may be summarized as follows:

a. Very young mycorrhizas contain a far larger amount of phosphorus than the normal roots.

	Colour given by the reagent.			
Portions examined.	Normal root.	Very young mycorrhiza.	Middle-aged mycorrhiza.	Old mycorrhiza
Fungous mantle.		green—deep green.	light green.	light greenish yellow.
Cortex.	light greenish yellow.	light green—green.	light greenish yellow.	light yellowish.
Central cylinder	light greenish yellow–light green.	light green—green.	light greenish yellow.	light yellowish.

b. The amount of phosphorus contained in the fungous mantle of the young mycorrhiza is larger than that in the fungous mantle of the old one.

These results seem to indicate that very young mycorrhiza can supply a larger amount of phosphorus to the host plant than the normal root absorbs.

(10) *Nitrate.*

Reagent: diphenylamine-sulphuric acid.

Two kinds of roots were selected. The one was large roots, 1·8–2·3 mm. in diam., which bore numerous mycorrhizas as their side-branches, and the other was roots of the same thickness which had many uninfected side-branches. The tests proved very clearly that the latter contained far larger amounts of nitrates than the former.

Longitudinal sections of both long, uninfected side-branches and mycorrhizas were then investigated.

a. *Normal root.* The normal roots are almost devoid of nitrate at their growing point. It begins to appear in the roots at a little back from the apex, and increases gradually toward the basal portion.

b. *Mycorrhiza.* The root tissue of the mycorrhiza contains almost no nitrates, while the fungous mantle has much of them. Perhaps nitrates may be used up by the fungous tissue before they reach the root tissue.

(11) *Potassium (and ammonium).*

Method employed: Molisch's sodium-cobalt nitrite method.

a. *Normal root.* The normal root has a large amount of potassium (and ammonium) even in the tissue of the growing point, and it increases little by little towards the older portion. The amount of it in the central cylinder more or less surpasses that in the cortex.

b. *Mycorrhiza.* The fungous mantle has a rather large amount of it, while the cortex and the central cylinder have a very small amount of it.

These tests show that (1) the normal root has a larger amount of potassium (and ammonium) than the root tissue of the mycorrhiza, (2) the host plant can absorb a tolerable amount of potassium (and ammonium) from the mycorrhiza.

2. *Normal roots and Boletus bovinus-mycorrhizas of Pinus densiflora.*

For the microchemical investigation I used the following six kinds of material:

a. *Normal roots.* The young uninfected root has white apex, indicating that it is continuously growing. It being very difficult to obtain uninfected side-branches, usually large roots, 0·11—0·40 mm. in diam., were used for the tests.

b. *Infected large roots.* There are large roots which have been enveloped by thin fungous mycelium without presenting the appearance of usual mycorrhizas.

c. *Large mycorrhiza.* Sometimes there are large roots which have been transformed into stubby mycorrhizas. They have the same structure as the usual small mycorrhizas.

d. *Young small mycorrhizas.* The mycorrhizas which have still no HARTIG's network.

e. *Middle-aged mycorrhiza.* The mycorrhizas which have more or less or tolerably developed HARTIG's network.

f. *Old Mycorrhiza.* The mycorrhiza which has both fully-developed HARTIG's network and the intracellular mycelium.

(1) *Amino-acids.*

Reagent: 1 % solution of ninhydrin. Cross or longitudinal sections of the fresh material were used for the investigation. The reagent began to give a violet colour to the material in 30 minutes, and the colour attained to its maximum in 1·5—2 hours at the room temperature, 31°C.

The results may be summarized as follows (Table 11):

a. In the normal root, a large amount of amino-acids is found in the growing point, but the amount decreases basipetally.

b. The amount of amino-acids contained in the root is gradually diminished by the fungous infection.

c. The root tissue of the mycorrhiza contains less of them than the normal roots.

TABLE 11.

Portions examined. / Normal root and mycorrhiza examined.	Colour given by the reagent. (o indicates no violet colour appeared). Fungous mantle.	Apical portion.	Basal portion.
Normal root.		deep violet.	very light violet.
Infected large root.		violet.	o
Large mycorrhiza.	light violet.	light violet.	o
Young small mycorrhiza.	light violet.	violet.	o
Middle-aged small mycorrhiza.	light violet.	light violet.	o
Old small mycorrhiza.	very light violet.	o	o

(2) *Mucilagenous substances.*

Reagent: ruthenium-red.

The mycorrhizal root has much mucilagenous substance in the cortex and more in the central cylinder. The amount increases gradually basipetally until all the vessels in the central cylinder are filled up with it, while almost no such feature is found in the normal root.

The fungous mantle and the enveloping mycelium gain a red colour by treatment with the reagent, indicating that they have much glycogen.

(3) *Phosphorus.*

Reagent: FRESENIUS' solution and 2 % phenylhydrazine chloride.

The existence of phosphorus in the cortex of the mycorrhizal root is quite uncertain as it turns to a dark colour by treatment with the reagent.

The results obtained are shown as follows (Table 12):

a. The root-tissue of the very young mycorrhiza contains a larger amount of phosphorus than the normal root.

b. The root-tissue of the middle-aged and old mycorrhizas contains, however, less phosphorus than the normal root.

TABLE 12.

Portions examined. / Normal root and mycorrhiza examined.	Colour given by the reagent.		
	Fungous mantle.	Cortex.	Central cylinder.
Normal root.		brownish yellow—brownish green.	green.
Very young small mycorrhiza.	blue—green.		bluish green—green.
Middle-aged small mycorrhiza.	brownish yellow—light greenish yellow.		light yellow—light greenish yellow.
Old small mycorrhiza.	brownish yellow.		brownish yellow—light yellow.

(4) *Sugar.*

Methods employed: (a) MEYER'S method and (b) *α*-naphtole-sulphuric acid.

a. MEYER'S method. The results obtained are shown as follows (Table 13):

TABLE 13.

Portions examined.	Colour given by the reagent.				
	Normal root.	Infected large root.	Large mycorrhiza.	Young small mycorrhiza.	Middle-aged small mycorrhiza.
Apical portion.	reddish brown.	light reddish brown.	light reddish brown.	light reddish brown.	yellowish.

b. *α-naphtole-sulphuric acid.* The meristematic portion of the normol and infected large roots gave a purple colour, though the colour in the latter was more or less lighter than that in the former.

In the mycorrhiza, the meristematic portion gave a light purplish colour. The fungous mantle gave a violet colour as in the case

of *Alnus*-mycorrhiza.

The results may be summarized as follows:

a. The normal root has a lager amount of sugar than the infected large root and the large and small mycorrhizas.

b. Sugar in the meristematic portion of the root is gradually diminished by the fungous infection.

(5) *Nitrate.*

Reagent: diphenylamine-sulphuric acid.

a. *Normal root.* The growing point of the normal root is almost devoid of nitrate. It begins to appear in the cortical tissue at a portion a little back of the apex and increases basipetally.

b. *Mycorrhizas.* The root-tissue of the mycorrhiza contains no nitrate, while the fungous mantle has a small amount of it.

(6) *Potassium (and ammonium).*

Method employed: Molisch's sodium-cobalt nitrite method.

a. *Normal roots.* The normal root has a large amount of potassium (and ammonium) even at the growing point. The amount increases basipetally until precipitated granules of cobalt-sulphide fill up almost half of the cell-cavity. The root hairs have numerous precipitations. This fact shows that there is great quantity of potassium (and ammonium) in the soil.

b. *Mycorrhizas.* The mycorrhizas contain such a large amount of potassium (and ammonium) in the root tissue that occasionally the amount surpasses that in the normal root.

3. *Normal roots and Hydnum affine-mycorrhizas of Pinus densiflora.*

The young mycorrhizas of *P. densiflora* caused by *Hydnum affine* and the mycelium woven by their projecting hyphae were tested with

various reagents shown as follows :

(1) *Amino-acids.*

a. *Mycelium.* The mycelium contains a small or sometimes a very small amount of amino-acids in it.

b. *Mycorrhiza.* The mycorrhiza contains almost no amino-acids in the root tissue.

(2) *Phosphorus.*

a. *Mycelium.* A very small amount of phosphorus is found in the mycelium.

b. *Mycorrhiza.* The amount of phosphorus contained in the root-tissue of the mycorrhiza is far smaller than that in the normal root.

(3) *Sugar.*

a. *Mycelium.* A large amount of glycogen is found in the mycelium.

b. *Mycorrhiza.* Almost no sugar is found in the root tissue.

(4) *Potassium (and ammonium).*

a. *Mycelium.* A very small amount of potassium (and ammonium) is found in the mycelium.

b. *Mycorrhiza.* The mycorrhiza contains almost no potassium (and ammonium) in the root-tissue.

The results may be summarized as follows :

a. The nutrients for the growth of the mycelium of *Hydnum affine* are obtained chiefly from the root-tissue of *P. densiflora.*

b. The mycorrhiza does not seem to supply phosphorus, potassium (and ammonium) to the host plant.

4. *Normal roots and mycorrhizas Form B of Abies firma.*

The morphological characteristics of the mycorrhiza Form B of *Abies firma* were described in my previous paper (Masui, 1, 1926).

It occurs in large numbers in wood-straw and is usually large and straight, so that it is easily sectionable. The materials used for the tests were as follows:

a. *Normal roots.* They have a white or yellowish apex and abundant root-hairs in the older portion.

b. *Large mycorrhizas.* There are numerous large roots which have been transformed into mycorrhizas. They bear usually several or many small mycorrhizas as their side-branches. Renewed growth pushing aside the living or dead fungous mantle is frequently found among them.

c. *Small mycorrhizas.* The side-branches are transformed into small mycorrhizas. They have the same structure as the large ones.

(1) *Amino-acids.*

Reagent: 1 % solution of ninhydrine.

The results obtained may be summarized as follows (Table 14):

a. The normal root has a larger amount of amino-acids than the mycorrhizal root.

b. Amino acid found in the young mycorrhizas are gradually exhausted with age.

c. The mycorrhizal roots, which can grow further, have still a large amount of amino-acids at their apical portion.

TABLE 14.

Root and mycorrhizas examined. / Portions examined.	Grades of colour given by the reagent. (10 indicates maximum grade).			
	Meristematic portion.	2 mm. back from the apex.		
		Cortex.	Central cylinder.	
			Outer portion.	Inner portion.
Normal root.	10	2—3	7—9	3
Young large mycorrhiza.	7	2—3	0—2	
Young large mycorrhiza with renewed growth.	7—8	3—4	5—7	3
Old large mycorrhiza with renewed growth.	7—9	0	5—7	3—4
Old large mycorrhiza.	0	0	0	0
Young small mycorrhiza.	2—4	0—1	0—1	
Old small mycorrhiza.	0	0	0	0

(2) *Phosphorus.*

Reagent: FRESENIUS' solution and 2 % solution of phenylhydrazine chloride.

The results obtained were as follows (Table 15):

TABLE 15.

Root and mycorrhizas examined. / Portions examined.	Colour given by the reagent.		
	Fungous mantle.	Cortex.	Central cylinder.
Normal root.		light greenish yellow.	light blue.
Young large mycorrhiza.	light yellowish green.	light yellow—light greenish yellow.	light greenish yellow—light green.
Middle-aged large mycorrhiza.	light yellowish green.	brown—yellow.	light greenish yellow—light green.
Old large mycorrhiza.	light yellowish.	brownish.	brownish—yellowish.
Young small mycorrhiza.	light yellowish green.	light greenish yellow.	very light green.
Old small mycorrhiza.	light yellowish.	brownish.	brownish.

These tests show that the normal root contains a larger amount of phosphorus than the mycorrhizal root.

(3) *Sugar.*

Method employed: (a) MEYER's method; (b) *α*-naphtole-sulphuric acid.

a. The results obtained by MEYER's method were as follows (Table 16):

TABLE 16.

Normal root and mycorrhizas examined. / Portions examined	Grades of colour given by the reagent. (10 indicates maximum).		
	Meristematic portion.	2 mm. back from the apex.	
		Cortex.	Central cylinder.
Normal root.	10	0	3
Young large mycorrhiza.	10	2—3	0
Large mycorrhiza with renewed growth.	7—9	2—3	0
Middle-aged mycorrhiza.	1—2	0	0
Young small mycorrhiza.	5—6	2—3	2—3
Middle-aged small mycorrhiza.	1—2	0	0

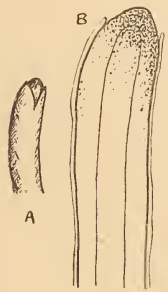

Fig. 91. *A*, a large mycorrhiza which has renewed its growth; *B*, a longitudinal section of *A*, treated with MEYER's reagents. Dots indicate sugar.

b. *α*-naphtole-sulphuric acid gave almost the same result with the normal root and the mycorrhiza as MEYER's method.

The results obtained may be summarized as follows:

a. The normal root has a larger amount of sugar than the mycorrhizal root.

b. The sugar contained in the young mycorrhiza is gradually diminished with age.

c. The mycorrhizal root, which

can grow further, has still a large amount of sugar at its apical portion (Text-fig. 91).

(4) *Starch.*

In order to compare the amount of starch in both kinds of the material, I chose, for convenience, two normal roots of different thikness and a very large mycorrhiza, 26 mm. long 0·42 mm. in diam., which bore 19 small mycorrhizas. The results obtained were as follows (Table 17):

TABLE 17.

Distance from the apex. mm.	Amount of starch in a root, 3 mm. in diam.	Amount of starch in a root, 0. 35 mm. in diam.	Amount of starch in a mycorrhiza, 0. 42 mm. in diam.
2	+	0	0
3	++	+?	0
5	++++	+	0
10	++++	+	0
15	++++	++	0
20	++++	++	+?
25	++++	+++	+

The small mycorrhiza is entirely devoid of starch in the root tissue. Even in the very large mycorrhizas, there is only a very small amount and only at their basal portion. In the case of the normal root, the large ones have always more starch than the smaller ones.

(5) *Glycogen.*

The fungous mantle of the mycorrhiza has a large amount of glycogen.

(6) *Potassium (and ammonium).*

By MOLISCH's sodium-cobalt nitrite method the fungous mantle, the

cell-wall of the cortical tissue and tannic substances gave a dark brown colour, in both kinds of the material, while the cell-wall in the central cylinder remained almost uncoloured. I could observe dark-coloured granular precipitations of cobalt sulphide only in the central cylinder, even though very thin hand-sections were used exclusively for the investigations.

a. *Normal root.* The meristematic portion of the normal root contains almost no potassium (and ammonium). It begins to appear in a root at a point slightly back of the apex, increasing in amount basipetally. The root hairs have much of it.

b. *Mycorrhizal root.* The mycorrhizas have a small amount of potassium (and ammonium) in the central cylinder. In the older mycorrhizas, sometimes, no granular precipitations of cobalt sulphide are found in the central cylinder.

The amount of potassium (and ammonium) in the normal root always surpasses that in the mycorrhizal root.

(7) *Nitrates.*

The normal root has nitrates in the cortical tissue as described in my previous paper (Masui, 1, 1926). But the mycorrhiza has almost no nitrates in its root-tissue.

(8) *Tannic substances.*

The tissue of the normal root as well as of the mycorrhiza has a very small amount of tannic substances, and the amount of them in the former is almost undistinguishable from that in the latter.

5. *Normal roots and compound mycorrhizas Form A*[1] *of Quercus pausidentata.*

I found a large number of uninfected roots and compound mycorrhizas at Kasuga-yama, Nara-Prefecture, on the 5th of August, 1926.

1. Masui, 3, 1926.

The materials used for the investigation were obtained there from a stock of these trees.

(1) *Amino-acids.*

a. *Normal roots.* The results obtained are shown in the following table (Table 18) :

TABLE 18.

Portions examined.	Colour given by the reagent.	
	Cortex.	Central cylinder.
Meristematic portion.	dark violet.	dark violet.
3 mm. back.	light violet.	violet.
5 mm. back.	very light violet.	light violet—violet.

b. *Mycorrhizas.* The results obtained are shown in the following table (Table 19) :

TABLE 19.

	Portions examined.	Colour produced by the reagent. O indicates no bluish colour appeared.			
		Fungous mantle.	Epidermis & Hartig's network.	Cortex.	Central cylinder.
Young mycorrhiza.	Apical portion.	violet.	Violet.		
	Basal portion.	light violet.	light violet.	very light violet.	light violet.
Middle aged mycorrhiza.	Apical portion.	light violet.	light violet.		
	Basal portion.	light violet.	very light violet.	o	very light violet.
Old mycorrhiza.	Apical portion.	o	o		
	Basal portion.	o	o	o	o
Heavily infected rootlets. (Young stage)	Apical portion.	violet.	violet.		
	Basal portion.	light or very light violet.	light or very light violet.	o	light or very light violet.
Heavily infected rootlet. (Middle-aged)	Apical portion.	light violet.	light violet—violet.		
	Basal portion.	o	o	o	o

c. *Compound mycorrhizas.* The very young tubercles have a large amount of amino-acids in every portion. In the fully developed ones the root-tissue of each mycorrhiza as well as the outer layer of the cortex-of-the-tubercle have a small amount of amino-acids, while the intermycorrhizal mycelium has still a large amount of them.

These tests clearly show that (a) the normal root has a larger amount of amino-acids than the mycorrhizal roots, (b) the root-tissue of the mycorrhiza gradually loses amino-acids with age and (c) the amount of them contained in the cortex-of-the-tubercle is less than that in the intermycorrhizal mycelium.

(2) *Phosphorus.*

The results obtained are shown in the following table (Table 20):

TABLE 20.

	Portions examined.		Colour given by the reagent.
Normal root.	Cortex.		light greenish yellow.
	Central cylinder.		light greenish yellow—greenish yellow.
Young tubercle.	Cortex of the tubercle.		light greenish yellow—light green.
	Intermycorrhizal mycelium.		light greenish yellow—light green.
	Mycorrhiza.	Epidermis & cortex.	light yellow.
		Central cylinder.	yellowish brown.
	Heavily infected rootlet.	Fungous envelope.	light greenish yellow—light green.
		Root-tissue.	yellowish brown.
Middle-aged tubercle.	Cortex of the tubercle.		light greenish yellow—greenish yellow.
	Intermycorrhizal mycelium.		light greenish yellow.
	Root-tissue of the mycorrhiza.		yellowish brown.
	Heavily infected rootlet.		no greenish colour appeared.

These tests show that (a) the normal root has a far larger amount of phosphorus than the mycorrhizal root and (b) the young tubercles contain a larger amount of phosphorus than the middle-aged or old ones.

(3) *Sugar.*

The result obtained are shown in the following tables :

a. MEYER'S *method* (Table 21).

TABLE 21.

		Colour given by the reagent. (o indicates no reddish brown colour appeared).		
	Portions examined.	Meristematic portion.	Basal portion.	
			Epidermis & cortex.	Central cylinder.
Normal root.		dark reddish brown.	dark reddish brown.	dark reddish brown.
Tubercle.	Axial root.		yellow—yellowish brown.	reddish brown—deep reddish brown.
	Mycorrhiza.		light yellow—light brown.	light brown—reddish brown.
	Heavily infected rootlet.		o	o

b. *α-naphtole-sulphuric acid* (Table 22).

TABLE 22.

		Colour given by the reagent. (o indicates no purple colour appeared).	
	Portions examined.	Epidermis & cortex.	Central cylinder.
Normal root.		deep purple.	deep purple.
Tubercle	Young mycorrhiza.	light purple.	purple.
	Old mycorrhiza.	o	o
	Heavily infected rootlet.	o	o

The central cylinder of the axial root as well as of the large mycorrhizas found around the axial root has a large amount of sugar, while smaller ones which exist at the exterior portion of the tubercle have a small, or a very small amount of it. In each case the epidermis and the cortex of the root contain a very small amount. As soon as the rootlets are enveloped by thick mycelium, they lose sugar entirely.

In short, (a) the normal root has a far larger amount of sugar than the mycorrhizal root and (b) the rootlets lose sugar because of the fungous infection.

The interesting thing about this mycorrhiza is that the tannic substances give the reaction of sugar.

(4) *Potassium (and ammonium).*

a. *Normal root.* The normal root has a large amount of potassium (and ammonium) even at the meristematic portion. By the tretament with the reagent, numerous large granules of cobalt sulphide, 4—21 μ in diam., were precipitated in the cells of the central cylinder. The number of the granules gradually increases basipetally.

b. *Mycorrhizas.* The root-tissue of the mycorrhiza has a small number of small granules, 1·5—7 μ in diam., in the epidermis, cortex and central cylinder. The fungous mantle and the intermycorrhizal mycelium have a slightly larger amount of it than the root-tissue.

c. *Heavily infected rootlets.* The mycelium found around the demolished rootlets has a large number of small granules of cobalt sulphide.

All this proves that the normal root has a far larger amount of potassium (and ammonium) than the mycorrhizal root.

(5) *Tannic substance.*

Reagent: iron sulphate. The tannic substances are found in mass

within the cell-cavity as well as contained in the cell-wall.

a. *Tannic substance in cell-wall* (Table 23).

TABLE 23.

Portions examined.	Colour given by the reagent.	
	Epidermis & cortex.	Central cylinder.
Normal root.	deep blue.	green—blue.
Mycorrhiza.	green.	

b. *Tannic substance in cell-cavity* (Table 24).

TABLE 24.

Portions examined.	Amount of tannic substances.		
	Epidermis and cortex except endodermis.	Endodermis.	Central cylinder.
Normal root.	++	++++	+
Young mycorrhiza.	+	++++	++
Old mycorrhiza.	o	++++	++

c. *Tannic substance in the tubercle* (Table 25).

TABLE 25.

	Portions examined.		Colour given by the reagent.
Middle-aged tubercle.	Cortex of the tubercle.		light green.
	Intermycorrhizal mycelium.		green—blue.
	Heavily infeced rootlet.	Thick mycelial envelope.	light green—light blue.
		Central cylinder.	green—blue.
Old tubercle.	Heavily infected rootlet.	Thick mycelial envelope.	no blue colour appeared.
		Central cylinder.	no blue colour appeared.

The results obtained may be summarized as follows:

a. The epidermis and the cortex of the normal root have a larger amount of tannic substances than those portions of the mycorrhizal root.

b. It is clear that the mycelium sucks off tannic substances from the root-tissue of the mycorrhiza.

6. *Normal roots and mycorrhizas of Pinus silvestris.*

Mycorrhizas Forms A and B obtained from young plants, 4 years old, were used for the test.

Usually longitudinal sections of Form B and total preparations of Form A were used, as the latter was too slender to cut into sections.

(1) *Amino-acids.*

a. *Normal root.* The normal root has a large amount of amino-acids at its meristematic portion, as in the plants mentioned above.

a. *Mycorrhizas Form A.* Usually the mycorrhiza of this type have a small amount of amino-acids at their apical portion. Some mycorrhizas have a very small amount or almost none even at their apical portion.

c. *Mycorrhizas Form B.* The mycorrhiza has a small amount of amino-acids at its apical portion, and the amount decreases basipetally. In the old mycorrhizas, I could not find even a trace of them in the root-tissue.

These tests clearly prove that amino-acids are gradually diminished by the fungous infection.

(2) *Phosphorus.*

The normal root contains a rather large amount of phosphorus in the root-tissue. But the mycorrhizal root has usually a smaller amount of it than the normal root. We come to the conclusion, therefore, that a

larger amount of phosphorus is not supplied by the mycorrhiza to the host plant than the normal root can usually absorb.

(3) *Sugar.*

The total preparations were tested with MEYER's reagent. The normal root has a large amount of sugar at its meristematic portion, while the two kinds of the mycorrhizas have a small amount of it.

The microchemical investigation shows that the infecting fungus sucks off amino-acids and sugar from the host root. Sometimes I found that the apex of an infected root, bearing many fresh mycorrhizas, had perished, even though that portion was free from the fungous infection. This may chiefly due to the fact that food substances have been sucked off by the infected fungus in the course of transmission from the mother root.

7. *Normal roots and cluster-type mycorrhizas of Pinus densiflora cultivated in sand and humous soil respectively.*

In order to see the difference between roots cultivated in sand and in humous soil respectively, young plants, sown in large pots two and half years ago and cultivated in both kinds of soil, were tested microchemically. The stems of the plants in sand soil had attained a length of 8—11 cm. those in the humous soil 12—15 cm. In examination of the roots I found that the former had a far larger number of mycorrhizas than the latter.

(1) *Amino-acids.*

a. *Material from humous soil.* Uninfected main root and the side-branches contain a large amount of amino-acids in their meristematic portion as well as in the central cylinder.

The young mycorrhiza has tolerable amount of amino-acids in the apical portion, and the amount gradually decreases basipetally. The old mycorrhiza has almost no amino-acids in the root-tissue.

b. *Material from sand soil.* The difference in the amount of amino-acids between the normal and the mycorrhizal root obtained from the sand soil was almost the same as in the case of those of the humous soil.

(2) *Sugar.*

a. *Material from humous soil.* Uninfected main roots and the side-branches have a large amount of sugar in their meristematic portions.

The mycorrhizal roots have a large amount of sugar when they are young, while it entirely disappears with age.

b. *Material from sand soil.* The difference in the amount of sugar between the normal and the mycorrhizal root obtained from the sand soil was almost the same as in the case of the humous soil.

The results may be summarized as follows:

a. The normal root has a larger amount of amino-acid and sugar than the mycorrhizal root.

b. Amino-acids and sugar contained in the root-tissue of the young mycorrhiza are gradually diminished with age.

c. There is no difference in amount of amino-acids and sugar between the mycorrhizas formed in humous soil and those in sand soil.

8. *Buttons of Armillaria Matsudake, Boletus bovinus and Cortinarius sp. (a).*

The materials used for the tests were as follows: *Armillaria Matsudake*, 3·2 cm. long and 1·5 cm. wide; *Boletus bovinus*, 10—12 mm.

long and 3—3·5 mm. wide; *Cortinarius sp. (a)*, 10—15 mm. long and 3—4 mm. wide.

(1) *Amino-acids.*

By the treatment with the reagent, primordial pilei and upper portion of the primordial stalks of three kinds of material gave a deep violet colour, while the basal portions of their stalks were violet, indicating that these buttons have a large amount of amino-acids (Text-figs. 92, *A* and 93, *A*).

(2) *Albuminous substance.*

By the treatment with MILLON's reagent, sections of each button equally gave a reddish brown colour (Text-figs. 92, *B* and 93, *B*).

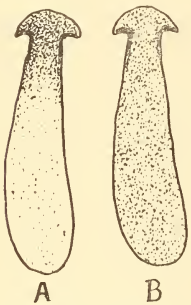

Fig. 92. Longitudinal section of buttons of *Boletus bovinus*. *A*, material treated with ninhydrin; *B* that treated with MILLON's reagent.

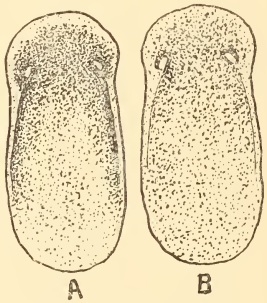

Fig. 93. Longitudinal section of buttons of *Armillaria Matudake*. *A*, material treated with ninhydrin; *B*, that treated with MILLON's reagent.

(3) *Phosphorus.*

By the treatment with the reagent, sections of each button evenly gave a blue colour.

(4) *Glycogen.*

Every portion of each button has a large amount of glycogen.

(5) *Potassium (and ammonium).*

Every portion of each button has a large amount of potassium (and ammonium).

In a word, buttons of *Armillaria Matsudake*, *Boletus bovinus* and *Cortinarius sp. (a)* have a large amount of amino-acids, albuminous substances, phosphorus, glycogen and potassium (and ammonium).

C. General results of the Microchemical Investigations.

The results obtained in the foregoing microchemical tests may be summarized as follws (Table 26):

1. *Amino-acids.* All the normal roots contains a large amount of amino-acids in the tissue of the growing point. When they are infected by any mycorrhizal fungi, the amino-acids are gradually diminished until almost none is found in the root-tissue. In all the cases investigated, therefore, the normal root contains more amino-acids than the mycorrhizal root.
2. *Carbohydrates.* Sugar is found also in large amount in the growing point of the normol root, and is gradually diminished by the fungous infection as in the case of amino-acids.

   Glycogen is usually found in large amount in the tissue of the fungous mantle, hyphal bundles as well as myeelium woven by numerous projecting hyphae of the mycorrhizas.

   The mycorrhiza Form B of *Abies firma* has almost no starch grains in the root tissue while the normal root has always a large amount of it in the central cylinder.
3. *Phosphorus.* In the case of the obligate mycorrhiza-formers usually the mycorrhizal root has a smaller amount of phosphorus than the normal root. But the young mycorrhizas of *Pinus densiflora* caused by *Boletus bovinus* as well as of *Alnus*

TABLE 26.

Material used. / Subst. tested.	*A. japonica* & its mycorrhiza Form A.	*P. densiflora* & its mycorrhiza with *B. bovinus*.	*P. densiflora* & its mycorrhiza with *H. affine*.	*A. firma* & its mycorrhiza Form B.	*Q. pausidentata* & its mycorrhiza Form A.	*P. silvestris* & its mycorrhizas.	*P. densiflora* & its cluster type-mycorrhizas.
Mucilage.	M > N	M > N					
Tannic subst.	M > N			M = N	M < N		
Amino-acids.	M < N	M < N	M < N	M < N	M < N	M < N	M < N
Albumin.	M = N						
Sugar.	M < N	M > N	M < N	M < N	M < N	M < N	M < N
Starch.				M < N			
Nitrates.	M < N	M < N		M < N			
Potassium (and ammonium).	M < N	M ≦ N	M < N	M < N	M < N		
Phosphorus.	M > N	M > N	M < N	M < N	M < N	M < N	

M indicates the mycorrhizal root and N the normal root.

japonica caused by *Cortinarius sp. (a)* have a far larger amount of it than the normal root, while in the middle-aged or the old ones, on the contrary, their root-tissue contains a smaller amount than the latter.

4. *Potassium (and ammonium).* Usually both the normal and the mycorrhizal root have a large amount of potassium (and ammonium) in the tissue. But the former has more than the latter, except in a case of *Pinus densiflora-Boletus bovinus* mycorrhiza which sometimes has more of these substances than the normal root.
5. *Tannic substances.* It has hitherto been considered to be a common fact in mycorrhizas that the mycorrhizas contain a larger amount of tannins than the normal roots. But evidence which indicates the contrary relation is given by the compound mycorrhiza Form A of *Quercus pausidentata* and mycorrhiza Form B of *Abies firma*. An interesting thing about the root of *Quercus pausidentata* is that the tannins, which are regarded especially as being glucosides, are gradually sucked off by the infecting fungus.
6. Young fruiting bodies of *Armillaria Matsudake*, *Cortinarius sp. (a)* and *Boletus bovinus* contain a large amount of amino-acids, glycogen, phosphorus and potassium in their tissues.

V. DISCUSSION.

The work presented in this paper may be discussed under six heads: (A) the structure of mycorrhizas in general; (B) the specific nature of mycorrhizal fungi; (C) the origin of the infecting fungi; (D) the development of mycorrhizas; (E) the seasonal relation of mycorrhizas; (F) the physiological relation of mycorrhizas.

A. The Structure of Mycorrhizas in General.

McDOUGALL (1914) reported that the mycorrhiza of *Tilia americana* is ectotrophic with occasionally endotrophic filaments. He gave the term "heterotrophic mycorrhiza" to such a one. MELIN (1923) believes that such endotrophic filaments are only haustorial hyphae which may be found in many ectotrophic mycorrhizas, and simply rejects the term "heterotrophic" as misleading (l. c. p. 108). Mycorrhizas of *Abies firma* and *Abies Mayriana* caused by *Cantharellus floccosus* (Masui, 1, 1926) however are ectotrophic with abundant endotrophic filaments which are not digested away by the host cells, and clearly differ from the usual ectotrophic or ecto-endotrophic mycorrhizas. It seems therefore very appropriate to call such a mycorrhiza heterotrophic.

The number of such heterotrophic mycorrhizas hitherto known is rather small but, in my opinion, there are many mycorrhizas which present the heterotrophic nature even if only slightly. When the mycorrhizas are young, they would typically show the ectotrophic structure. But with age occasionally the filaments which constitute the intercellular mycelium enter into the cell-cavity of the host cell dissolving the cell-wall. Examples of this may be found not only in several mycorrhizas described in the present paper but also in the mycorrhiza Form B of *Abies firma* and the compound mycorrhiza of *Quercus pausidentata*.

In vitro, the mycorrhizal fungus usually transforms the roots of vascular plants into heterotrophic mycorrhizas or sometimes into pseudo-mycorrhizas (MELIN 1923). Perhaps the heterotrophic nature may be emphasized by some conditions of the substratum.

Morphologically this mycorrhiza may very well be considered as an intermediate form between the true ectotrophic and the ecto-endotrophic mycorrhizas.

The ecto-endotrophic mycorrhizas of *Pinus Montana*, *Pinus silvestris* and *Betula* have been reported by TUBEUF (1903), MÜLLER (1023) MELIN (1923) and others. Also the *Pinus densiflora-Boletus bovinus*-mycorrhiza clearly belongs to this form, as is indicated by its characteristics, namely: (1) in external appearance it looks like an ectotrophic mycorrhiza, (2) usually the intracellular hyphae develop well within the cell-cavities sometimes filling them up with mycelium, and (3) the intracellular mycelium is ultimately digested away by the host cells (Text-figs. 11—15), as in the well-known cases of the endotrophic mycorrhizas of Orchids and others. They represent really intermediate forms between the heterotrophic and the endotrophic ones.

To put it briefly, it may be considered that there is a gradual transition between the true ectotrophic mycorrhizas and the endotrophic ones, with many kinds of heterotrophic and ecto-endotrophic mycorrhizas interposed.

MELIN (1923) states in the case of the bifurcated mycorrhiza type I (the ectotrophic mycorrhiza) of *Pinus silvestris* that (1) it is nearly constant in the tannin-sheath of the mycorrhizal root that very thin hyphae pass through the cells which have been filled up with tannic vacuoles, (2) the same hyphae are found also in the meristematic cells of the cortex, and in this case they degenerate before the HARTIG's networks are formed, and (3) the granular layer are filled up with granules of various size, measuring 1—2 μ, sometimes less than 1 μ in diameter. These granules are considered to be perhaps a sort of fungous excretion.

The author's investigation has shown, in the case of the bifurcated mycorrhizas of *Pinus densiflora* and *Pinus silvestris*, that the intracellular hyphae are found in quite rare cases in the cells of the tannin-sheath and in no case in those of the meristematic region (see p. 185—191). The very young cells of the cortex contain minute granules or more or less elongated bodies, 0·5—1 μ in diam., of tannic substances in the cytoplasm. Such bodies look as if they

were pieces of fungous filaments. The uninfected roots obtained from pines cultivated in sterilized soil in ERLENMEYER-flasks contain also such substances in the young cells of the cortex. It is clear therefore that they are ergastic bodies of the cell and not the infected fungus (Pl. VII, Fig. 2).

Fully-developed cortical cells enveloped or half enveloped by HARTIG's network contain abundant granules, measuring 1·5—3·2 μ in diam., or minute bodies, which are less than 1 μ in diam., in the central vacuoles. It is clear that these bodies are those found by MELIN in cells of the granular layer. The same substances are found also in cells of the corresponding portion of uninfected roots (Pl. VII Fig. 6—11), indicating that they are neither peculiar to the mycorrhiza nor a fungous excretion of it.

According to MELIN's description, the bifurcated mycorrhiza type II (the ecto-endotrophic mycorrhiza) of *Pinus silvestris* has a distinct digesting-layer which is made up of three inner cell-rows of the cortex, except the endodermis. The cells of this layer contain "hyaline bodies" of various form and size, measuring 2—3 μ or sometimes more than 5 μ in length. He believes that, in his own words, "die hyalinen Körper haben sich aus Pilzhyphen gebildet" and the host plants derive benefit from the digestion of them.

Mycorrhizas of both *Pinus densiflora* and *Pinus silvestris*, fixed with chromo-acetic or chromo-acetic-platic chloride solution and stained with vesuvin-aniline blue, acid fuchsin or FLEMMING's safranine-gentian violet-orange, contain abundant granules in the inner cortical cells. The morphological characteristics of them are exactly identical with those of the hyaline body described by MELIN (see p. 185—191 of this paper). In the living material, both the hyaline bodies and the tannic granules found in cells of the granular layer are identically hyaline ones with refringency. When the mycorrhizas are fixed with the above mentioned fixatives, these substances show a difference in their affinity for staining dyes according to the distance from the

surface of the material, namely, the granules in two or three cell-rows of the outer cortex are coloured by the staining dyes, while those in the inner layer are left uncoloured. Perhaps the latter have been fixed only with acetic acid which penetrates sooner than chromic acid. In my opinion these two kinds of granules are of the same substance and of tannic nature.

In the fixed and stained preparations of uninfected roots, the cells of the inner cortical layer also contain hyaline bodies, clearly indicating that these bodies are not endophytes.

In a word, the above mentioned bifurcated mycorrhizas of *Pinus densiflora* and *Pinus silvestris* have no digesting layer.

The *Boletus bovinus-Pinus densiflora*-mycorrhiza or the tubercle mycorrhiza clearly has endotrophic fungous filaments which are destined to be digested by the host cells. The filaments chiefly enter the cells of the first, second and third cell-rows of the cortex, and rarely in the cells which contain the so-called hyaline bodies.

Melin states that the tubercle mycorrhiza of *Pinus silvestris* has the digesting-layer made up of 5-6 cell-rows, and the cells of its inner region contain also the hyaline bodies. According to his description and illustration, it is quite reasonable that cells of the outer region of the digesting layer should have true endophytes. But granules found in cells of the inner layer are perhaps not pieces of endophytes but of tannic nature.

B. The Specific Nature of Mycorrhizal Fungi.

Since the year 1887, when Rees and Fisch published a paper, for the first time, dealing with the mycorrhizal fungi, more than forty species of ectotrophic-mycorrhiza-formers have been mentioned. With one exception, Elaphomyces, they are all Basidiomycetes, belonging to the following genera: *Amanita*, *Armillaria*, *Boletus*, *Cantharellus*, *Cortinarius*, *Geaster*, *Lactarius*, *Russula*, *Scleroderma* and *Tricholoma*.

Ten species now added by the author are also Basidiomycetes, and among them there are two new genera, *Hydnum*[1] and *Polyporus*.

The mycorrhizal fungi have acquired various habits and adapted themselves to different methods of nutrition. Some are usually saprophytes forming mycorrhizas occasionally. Others thrive only upon special substances, as for example, roots of some particular plants. In my opinion, the former are facultative mycorrhiza-formers and the latter obligate ones. Among the mycorrhiza-formers studied, *Tricholoma Shimeji*, *Boletus bovinus* and *Scleroderma vulgare*(?) belong to the former group and *Armillaria caligata*, *Armillaria Matsudake*, *Cantharellus floccosus*, *Hydnum affine* and *Polyporus leucomelas* to the latter. *Cortinarius* seems to prey chiefly upon living roots but also have an intimate connection with humus or wood-straw, indicating that perhaps these are the obligate mycorrhiza-formers inclined to the facultative side. Thus no hard and fast line can be drawn between these two classes.

Among many mycorrhiza-forming humous-Basidiomycetes there are two types, the fungi which are capable of being cultivated on an artificial medium and those which do not grow on it. MELIN cultivated many species of *Boletus*, one or more species of *Russula*, *Lactarius*, *Amanita*, *Cortinarius*, and *Tricholoma* on the artificial medium, and made the following statement with regard to the other fungi: "Die anderen Arten sind aus den auf Platten oder in Nährlösung gelegten Fruchtkörperstückchen nicht zum Wachsen zu bringen. Dies ist z. B. bei einer Reihe von untersuchten *Cortinarius*-, *Lactarius*-und *Russula*-Arten, ferner bei Arten der Hymenomyceten Gattungen *Cantharellus*, *Gomphidius*, *Inocybe*, *Hydnum* und *Hygrophorus* der Fall. Es erscheint mir wahrscheinlich, dass wenigstens die meisten von derjenigen Gruppe der Humus-Hymenomyceten, die sich nicht auf künstlichen Substrate kultivieren liessen, so obligate Mykorrhizasymbionten sind....."[2]

1. MELIN conjectured that *Hydnum* might be a mycorrhiza former, though he had found no actual example.

2. Emphasis my own.

I have succeeded in making pure cultures of *Boletus bovinus*, *B. luteus* (?), *Armillaria caligata*, *A. Matsudake*, *Scleroderma vulgare*(?), and *Tricholoma Shimeji* on the artificial medium, but *Cantharellus floccosus*, *Cortinarius cinnamomeus*, *C. sp. (a)*, *C. sp. (p)*, *C. sp. (k)*, *Hydnum affine* and *Polyporus leucomelas* even with repeated efforts did not grow on it.

Among the just mentioned fungi which have been cultivated, *Boletus*, *Scleroderma* and *Tricholoma* are facultative mycorrhiza formers. *Armillaria caligata* and *A. Matsudake* are, however, found only connected with living roots, indicating that they are obligate mycorrhiza formers, in spite of the fact that they can grow on the artificial medium. Thus it seems not very adequate to classify the mycorrhizal fungi into two groups according to their behaviour on the artificial medium only.

As to the interrelation of mycorrhizal fungi with host roots, there are various peculiarities on account of the activity of hyphae. Each fungus usually forms a definite mycorrhiza, differing from others, with roots of definite plants. Whether the structure of the mycorrhiza is ectotrophic, heterotrophic, or ecto-endotrophic, may chiefly be determined according to the specific nature of each mycorrhizal fungus. *Cantharellus floccosus* forms a heterotrophic mycorrhiza with roots of *Abies firma* as well as with those of *Abies Mayriana*. *Cortinarius cinnamomeus* transforms the rootlets of both *Pinus densiflora* and *Populus tremula var. villosa* into ectotrophic mycorrhizas with long projecting hyphae, and their growing large roots into heterotrophic ones. Melin states that a certain *Boletus* transforms the roots of *Pinus silvestris* into compound ecto-endotrophic mycorrhizas. So also in the case of *Pinus densiflora* and *Quercus pausidentata* so far as *Boleti* are concerned. These examples show therefore that when one fungus is concerned with mycorrhiza formation on two or more different plants, the same mycorrhizal form generally results on their roots, as the specific nature of the fungus usually plays a far greater rôle in the formation of the mycorrhiza, than that of the host plant.

Moreover, a thing important enough in considering the specific nature of mycorrhiza-formers is the relative strength of the intracellular hyphae of mycorrhizas against the vitality of host cells. The hyphae of *Cantharellus floccosus*, *Cortinarius cinnamomeus* and *Hydnum affine*, the mycorrhizal fungi of *Pinus densiflora*, strongly resist the host cells, remaining long in a vigorous condition within the cell-cavities. According to MELIN's opinion, these intracellular hyphae must be haustorial hyphae, as in these mycorrhizas no instance was observed in which the hyphae were digested away by the host cells. In the case of *Boletus bovinus-Pinus densiflora*-mycorrhiza, on the contrary, all the intracellular mycelium is digested away by the root, though no haustorial hyphae are formed, MELIN states, in the *Boletus-tuberele*-mycorrhiza of *Pinus silvestris* and the *Boletus*-ecto-endotrophic mycorrhiza of *Betula*, that the haustorial hyphae and the hyphae which are destined to be digested are found in the same mycorrhiza. I have not yet met with such cases.

McDOUGALL (1922) says that "the relatively thin and loosely constructed fungous mantle may be somewhat characteristic of mycorrhizas caused by a species of *Cortinarius*". The present author (4, 1926) has stated however that "not all the species of the genus *Cortinarius* always cause a similar mycorrhiza on the root of higher plants". The author's subsequent investigation shows moreover that not only the fungous mantle of mycorrhizas caused by *Cortinarius* but also that of mycorrhizas caused by the other fungi more or less differ in thickness as well as in the texture according to the difference of the fungi.

There is some modification in the thickness of the fungous mantle in each kind of mycorrhiza. MELIN believes that the modification follows from the difference of soil where the mycorrhizal fungi live, and makes the following remarks: "Die verschiedenartige Entwicklung des Mantels hängt.....damit zusammen, wie die Pilze im Boden gedeihen. Leben diese unter optimalen Bedingungen, dann entwickeln sie einen kräftigeren Mantel als sonst." In the case of the

facultative mycorrhiza-fomers, in my opinion, the soil may play some rôle in the development of the the fungous mantle, but in the obligate mycorrhiza-formers, which receive little benefit from soil, the modification may chiefly depend on the amount of nutrients contained in the roots on which they prey.

The specific nature of each mycorrhizal fungi is thus presented in its mycorrhizas. As each fungus differs in nature from the others, the resulting mycorrhiza seems to show a difference in structure as well as perhaps in physiological nature from the others, although the nature of the host root may have something to do with this.

C. The Origin of the Infecting Hyphae.

The problem of the fungous infection on young roots has been discussed by previous investigators. Frank and Müller beleave that roots of vascular plants are infected by the mycelium which is spreading through various layers of soil. I (1, 1926) have stated that the roots of *Abies* are infected by the mycelium of *Cantharellus floccosus* which develops from preexisting mycorrhizas as well as perhaps by the filaments of the same fungus germinated directly from spores.

My investigations upon both facultative and obligate mycorrhiza formers have shown that the mode of infection may differ in each case according to the nature of the fungus concerned. In the obligate mycorrhiza former such as *Armillaria caligata*, *A. Matsudake*, *Coritnarius cinnamomeus*, *C. sp. (a)*, *C. sp. (p)*, *C. sp. (k)*, *Hydnum affine* and *Polyporus leucomelas*, the same relation as in *Cantharellus floccosus* usually occurs, as they grow actually connected with living roots of host plants; whereas in the facultative ones, such as *Boleti* the mycelium usually existing as a saprophyte in soil may be concerned in the formation of new mycorrhizas.

Melin (1925) states that "in der Natur herrscht eine heftige Konkurenz zwischen verschiedenen Mykorrhizabildner um die Wurzeln." It

is conjecturable that such a phenomenon may occur in nature. However, the obligate mycorrhiza-formers, which form the so-called soil-mycelium on the surface of humus or wood-straw, such as *Armillaria caligata*, *A. Matsudake*, *Hydnum affine*, and *Polyporus leucomelas*, do not seem to compete much with other fungi for getting pine roots which grow through the mycelium itself.

D. The Development of Mycorrhizas.

The question, whether the inward infection or the fungous mantle is first formed in the infected roots, remains unsettled. FRANK (1885) and MCDOUGALL (1914) maintain that the mantle is formed first and then the lichen structure within the rootlets. MÖLLER's opinion (1903,) is just the opposite. I have stated in my previous paper that in the mycorrhiza of *Cantharellus floccosus* both cases may be observed. As the mycorrhiza is formed rapidly, it is difficult to obtain good microscopical specimens of successive stages, unless the materials are collected with that special object in view. In ordinary cases however the fungous mantle precedes the formation of HARTIG's network.

MÖLLER (1902) has reported about the endotrophic filaments in the large roots of the seedlings of *Pinus silvestris*. Not only the roots of seedlings, but growing large roots of *Pinus densiflora* and *Populus tremula var. villosa* have also the endotrophic filaments. According to MÖLLER's opinion (1903) the infection takes place through root-hairs extending from root-hair cells into adjacent cells, as has been shown by several authors in the case of the endotrophic mycorrhiza. But I have not yet met with such a mode of infection so far. In the above-mentioned two plants, one can distinguish two modes of infection: (1) the fungous filaments enter directly into the cell of the outermost row of the root-tissue; (2) they enter the intercellular spaces and then into the cell-cavity (Text-fig. 22). In these cases, clearly the inward infection precedes the formation of the fungous mantle.

I am forced to conclude therefore that in usual cases the formation of the fungous mantle precedes the inward infection, but in the growing large root the order is just the reverse. It is quite reasonable that different ideas should result from different kinds of materials.

E. The Seasonal Relation of Mycorrhizas.

Stahl (1900) believed that autumn is the season in which the utmost development of the mycorrhizal fungi occurs. Möller and McDougall also reported that they become most plentiful in September. Melin says briefly that "Die Periodizität der Mykorrhizabildung hängt offenbar mit dem periodischen Wurzelwachs überhaupt zusammen." It may be emphasized more precisely.

According to Büsgen (1901) the roots of many vascular plants show the most active growth during September and October. As a matter of fact the mycorrhizas are formed most plentifully in these seasons.

There are two conditions necessary for the formation of mycorrhizas: (1) the roots must be just growing, and (2) the infecting fungus must be presented in an active condition, as described by McDougall (1916). The new roots of *Alnus japonica* become plentiful during spring and summer, just coinciding with the period when the mycorrhizas (Forms A and B) are formed abundantly. The same relation is also observed between the roots of *Pinus densiflora* and all the kinds of its mycorrhizas.

The most interesting evidence is obtained from the mycorrhizas of *Populus tremula var. villosa* and *Pinus densiflora* which are formed by one and the same fungus, *Cortinarius cinnamomeus*. The fresh mycorrhizas of the former begin to increase in number in April and May in accordance with the growth of its roots, while those of the latter become plentiful during autumn, just coinciding with the season of the luxuriant growth of the pine roots. It is interesting to note that the mycelium of *Cortinarius cinnamomeus* exists during these periods in an active

condition.

McDOUGALL (1914) has stated that "the mycorrhizas are annual, but there is no time of year when no specimen at all can be found since the formation of new mycorrhizas usually begins before all of the old ones are dead". Indeed, the fungus exists throughout the seasons, even in mid-winter or in dry seasons, in more or less active condition. But the main occurrence is determined by the seasonal variation of the root growth, that is, there is a coincidence of seasons in which a greater number of new roots are formed and the mycorrhizas of the plant become plentiful, though the relation may be more or less shifted by some other factors.

F. The Physiological Relation of Mycorrhizas.

The physiological relation of mycorrhizas in woody plants may be discussed under the following heads: (1) the nutritive relation between mycorrhizal fungi and roots; (2) the nutritive relation between the fruiting bodies of mycorrhizal fungi and roots.

1. *The Nutritive Relation between Mycorrhizal Fungi and Roots.*

It is a well known fact that the morphological relation between the fungi and the roots in mycorrhizas is very intimate. Not only does the fungous mantle adhere fast to the root-tissue, but also the fungous filaments enter between the cortical cells of the root, forming the so-called HARTIG's network. In the latter case, the fungus is associated so intimately with the root-tissue that the mycelium looks as if it is continuous with the cortical cells. The nutrient solutions of the cell-sap can therefore easily pass from cell to cell through the walls.

a. *The Relation between the Mycorrhizal Fungi and the Nutrients transported to the growing points of roots.*

When the development of the mycorrhiza is observed carefully, one will find that at first the rootlets are enveloped usually by thin fungous mantles and later, in many cases, projecting hyphae or hyphal bundles are given off from the surfaces after the inhibition of growth of the mycorrhizal roots. These processes are recognized also in mycorrhizas exposed to damp air.

Microchemical tests make clear the nutritional relationship between the roots and the infecting fungi. Normal roots contain a very large amount of amino-acids and sugar at their growing point, which have been transported from the mother roots and are to be used for the further growth. When the mycorrhizas are very young, these nutrients are found still in a tolerable amount in the apical tissue, but they are diminished gradually due to the development of the fungous mantle. The projecting hyphae and the hyphal bundles grow most abundantly in the apical region of the root where larger amount of the nutrients is found than in any other portions. In the old mycorrhizas at last no nutrients are detectable in the root tissue, and the projecting hyphae show themselves to have lost their vitality. The growth of the projecting hyphae or hyphal bundles is retarded, therefore, somewhat parallel with the decrease of nutrients in the root-tissue. No more nutrient is retained generally for the further growth of the mycorrhizal root after it is completed. I have not yet met with cases where amino-acids and sugar are transported back to the mother root after the inhibition of growth.

The nutritive relation of the fungous mantle with humus or wood-straw is perhaps a point important to be discussed sufficiently. There are often mycorrhizas which have been formed entirely exposed to damp air. In such mycorrhizas, the nutrients of the root are gradually dimi-

nished in proportion to the development of the fungous mantle. As the fungous mantle is actually free from the humus, we may safely conclude in such a case that the material for the growth of the mantle is obtained entirely from the root-tissue. Similar examples may be presented also by pine mycorrhizas caused by *Armillaria caligata*, *A. Matsudake*, *Hydnum affine* and *Polyporus leucomelas*, they being found in large numbers associated within each soil-mycelium which contains little humus, as well as by *Quercus psusidentata*-compound mycorrhiza Form A which is composed of a large number of associated mycorrhizas and an enveloping mycelium, containing no humous particles.

From the foregoing descriptions we may conclude decidedly that the mycorrhizal fungus obtains the nutrients, for the development of the mantle, chiefly from the root-tissue of the mycorrhiza.

That the roots cease their further growth after they have been transformed into mycorrhizas seems, therefore, due to mechanical resistance, to a certain degree, but chiefly to the loss of such nutrients. The mycorrhizal fungus can live while the nutrients are kept in its root-tissue. The fact that most mycorrhizas are "annual" as stated by McDOUGALL, results without doubt from the death of both the fungus and root-tissue which follows sooner or later after the nutrients are all used up.

There are however occasionally some vigorous mycorrhizas, which grow anew even after their fungous mantles are completed. Microchemical investigation shows that such mycorrhizas still contain a tolerable amount of amino-acids and sugar at their growing point (compare p. 233–235).

The facultative mycorrhiza-formers usually live in humus or wood-straw. When such fungi are going to form mycorrhizas imbedded within humus or wood-straw, the fungous mantle may also obtain some kind of nutrients from them.

It has hitherto been believed that all the mycorrhizas contain a larger amount of tannic substance than the normal root. Accordingly it has been considered important for understanding the nature of the mycorrhiza

to elucidate this point.

Peklo (1909, p. 241) believes that, to put it in his own words, "Der Pilze vermag jedoch die Gerbstoffe in sich aufzunehmen und diese als Nährstoffquelle zu verwerten". Rexhausen (1920, p. 42) states that: "Der sich kenntlich machende Zucker war vielleicht glykosidisch an den Gerbstoff gebunden.....Soweit die gerbstoff-führenden Zellen überhaupt für die Ernährung des Pilzes in Betracht kommen, ist es sicher nur der gebundene Zucker, der als C-Quelle dient und nicht der Gerbstoff selbst".

Peklo (1913) found a large number of fungous hyphae in the cells of the endodermis of *Picea*-and *Pinus*-mycorrhiza, denoting it as "Pilzscheide".

Melin (1923) strictly denied, on the one hand, the existence of the hyphae in the endodermis of the same mycorrhizas, but found, on the other hand, haustorial hyphae in the outer tannin-cells, and made the following statement: "Zweifelsohne sind sie Haustorienhyphen, durch die der Pilzsymbiont von der Wirtpflanze gewisse Stoffe erhält".

My investigation shows that (1) not all kinds of mycorrhizas contain a larger amount of tannic substances than the normal root, (2), in many cases, tannic substances found in mycorrhizas do not seem to be glycosides (see p. 241–243) and (3) the intracellular hyphae are found in quite rare cases in the tannin-cells and in no case in the endodermal cells. The fact that tannic substances themselves are not the nourishment of ordinary mycorrhizal fungi, has been reported by Rexhausen. Melin (1925) shows also the same fact.

In my opinion, the mycorrhizal fungi absorb, in many cases, sugar which exists, not in the form of glycoside, in the meristematic portion of the root. I introduce here, however, mycorrhizas of *Quercus pausidentata*, as an exception, whose tannins are absorbed by the infected fungi, the tannins being, in this case, glycoside, as clearly shown in the microchemical tests.

b. *The Absorption of Water and Nutrient Salts through Mycorrhizas.*

The mycorrhizal roots of woody plants are found usually enveloped by the fungous mantle. The formation of root-hairs is thereby completely inhibited. A certain amount of water and essential salts must be absorbed, therefore, by the mycorrhizal roots through the fungous mantles. It must be remembered, however, that the mycorrhiza need not necessarily be the water supplier, if there are in the deeper layer of the soil, a large number of rootlets not infected by the fungus and suitable for the absorption of water, as in the case of fairly large trees.

The amount of nutrients to be absorbed seems to differ chiefly according to the kind of the mycorrhizas, the environmental conditions where they are produced, and also to the stage of development in each of them.

When *Boletus bovinus-Pinus densiflora*-mycorrhizas are very young, their root-tissue usually contains a far larger amount of phosphorus than the normal roots. But in those of middle or old age this relation becomes just the opposite. The same relation may be observed also in *Cortinarius sp. (a)-Alnus japonica*-mycorrhizas. In these cases it may well be considered that these mycorrhizas convey a larger amount of phosphorus to the host plants than their normal roots absorb. But another point important to mention is that this relation seems to be presented only for short period when the mycorrhizas are young. Later the direction of the translocation is inverted.

In the case of *Hydnum affine-Pinus densiflra*-mycorrhiza, on the contrary, the mycorrhizal root contains much smaller amount of phosphorus in every stage of its development than the normal root, indicating that the mycorrhiza supplies no phosphorus to the host plant (p. 232).

It is shown by the microchemical tests that a tolerable amount of potassium (and ammonium) is found in the root-tissue of mycorrhizas, though usually the amount is more or less smaller than in the normal

roots, indicating that the mycorrhiza is not a good supplier of potassium (and ammonium) compared with the normal root. The *Boletus bovinus-Pinus densiflora*-mycorrhiza is in this case an exception, as occasionally a larger amount of the substance is found in the root-tissue of the mycorrhiza than in the normal root.

The mycorrhiza Form A of *Alnus japonica* has a peculiarity which calls for special mentioning. The vessels in the basal portion of this form are entirely filled up with mucilage and tannic substances, so that the transportation of food substances seems difficult after the rootlets are transformed into mycorrhizas (p. 219).

c. *The Digestion of the Intracellular Hyphae.*

In many kinds of endotrophic mycorrhizas, most of the intracellular hyphae are digested by the host cells. The same fact has been found in cases of ecto-endotrophic mycorrhizas, while in the ectorophic and heterotrophic ones no such phenomenon occurs.

Melin states in the case of ecto-endotrophic mycorrhizas of *Betula* and *P. silvestris* caused by *Boletus*, that the root digests the particular fungous hyphae, or, as he calls them, "Eiweisshyphen" and so derive benefit from the association.

In this respect *Boletus bovinus-Pinus densiflora*-mycorrhiza, an ecto-endotrophic one, is particularly interesting, as the nutritive relation between the hyphae which are destined to be digested and the root can be traced precisely. The fungous mantle of this mycorrhiza, as already mentioned, is considered to take food not only from the root-tissue but also from the surrouding humus. The fungous mantle makes extraordidary development inward, pushing aside the cells of the first row of the cortex or the pseudoepidermis (Text fig. 11.). The intracellular hyyhae are found usually in the first and the second rows of the cortex. Therefore the fungous hyphae enter these cells almost directly from the inner surface of the fungous mantle, freely dissolving the cell-wall. It may well be considered now that so far as these hyphae are formed by the nutrients absorbed from the

surrounding humus, the root, in this respect, derives benefit from the association by the digestion.

2. *The Nutritive Relation between the Fruiting Bodies of Mycorrhizal Fungi and Roots.*

The mode of origin of fruiting bodies from the mycorrhizal fungi, though little attention has been paid to this hitherto, is very important in order to make clear the physiological relation between the roots of vascular plants and the fungi.

The fruiting body of *Hydnum affine, Armillaria caligata, A. Matsudake* and *Polyporus leucomelas* originates from the mycelial network or soil-mycelium interwoven by the hyphae projected from the surfaces of numerous mycorrhizas. Such mycelium contains almost no humous particles within it. Minute buttons of *Cortinarius ciunamomeus, C. sp. (a), C. sp. (p)* and *C. sp. (k)* are always produced directly from one or more mycorrhizas which have been connected with humus or wood-straw. Thus the morphological relation between the fruting bodies and mycorrhizas is very intimate.

The young fruiting body contains as essential food for the growth, as the microchemical tests show us, rather large amount of amino-acids, sugar, phosphorus and potassium (and ammonium) (see p. 245–247). But where do these nutrients come from? The author has reported that the fruiting bodies of *Cauthariellus floccosus* originate even from the infected roots of *Abies* which have been exposed to the air. In such a case all the nutrients used for the formation of the fruiting bodies are obtained directly from the root, as microchemical tests also prove. In the case of *Hydnum affine*, the mycelium, from which the fruiting bodies are produced, contains few humous particle, so that the fruiting bodies may not be originated, unless a large amount of the nutrients is supplied by the mycorrhizas or from other sources. Melin (1925) has stated that the mycorrhizal fungi as well as the mycorrhizas have no-

thing to do with the fixation of the atmospheric nitrogen. We are forced therefore to conclude that the fruiting bodies of *Hydnum affine* are originated chiefly at the expence of the mycorrhizal roots of *Pinus densiflora*. *Polyporus leucomelas*, *Armillaria caligata* and *A. Matsudake*, all of which form similar mycorrhizas on pine roots and also produce fruiting bodies in the same way as *Hydnum affine*, may have also the same physiological relation with the host plant.

In the case of the above mentioned four species of the genus *Cortinarius*, nutrients for the formation of fruiting bodies may be absorbed not only from the mycorrhizal root but also from surrounding humus or wood-straw.

The foregoing remarks may be summarized as follows:— *Boletus* supplies, in connection with the mycorrhiza formation, a certain amount of phosphorus and other nutrients to the host plants, while, on the other hand, it absorbs amino-acids and sugar from the roots. In the case of *Hydnum affine*, *Cantharellus floccosus*, *Polyporus leucomelas*, *Armillaria caligata* and *A. Matsudake*, on the contrary, not merely do they afford no benefit to the host roots but kill them. *Cortinarius* clearly shows the parasitic nature on roots in respect to the mycorrhiza formation as well as to the production of fruiting bodies, but seems to supply a tolerable amount of essential salts to the host plant at least at the beginning.

It is clear that all the mycorrhizas of woody plants do not show the same biological siginificance. The *Boleti* are considered, in the above mentioned respects, to associate more or less symbiotically with roots, so I may denote them as "hemi-symbionts": moreover, in my opinion, many other facultative mycorrhiza-formers may be also hemi-symbionts. The above mentioned species of *Hydnum*, *Cantharellus*, *Polyporus* and *Armillaria* are considered, on the contrary, to be root-parasites of *Pinus densiflora*. *Cortinarius*-mycorrhizas may be intermediate between the parasitic and hemi-symbiotic associations, though

more or less inclined to the parasitic side.

MELIN (1923) stated that "die Zellkerne in den vom Réseau umgebenen Rindenzellen erhalten sich sehr lange Zeit am Leben" as an evidence that such cortical cells are benefited by the intercellular mycelium. The cell-nucleus in the meristematic region of the mycorrhizal root, however, frequently lacks the nucleolus and moreover its structure is very rough compared with that of the normal root (Pl. VII, Figs. 1 and 8). When the cells are enveloped by HARTIG's network, all their nuclei lose nucleoli, forming an indistinct nuclear reticulum or dull granules (Pl, VII, Figs. 11—13). Accordingly it may be presumed that these nuclei show greatly decreased nuclear activities, though it is difficult to decide critically whether they are living or dead. When, however, such nuclei are compared with those in normal roots, one will find that cortical cells are not nourished by the intercellular mycelium.

As a second evidence of mycosymbiosis, MELIN stated further that the host plants derive benefit from the association with mycorrhizal fungi: "kann man.....auf jungen Waldboden.....auf denen vorher wegen der ökologischen Verhältnisse keine Mykorrhizapilze haben wachsen können, nebeneinander Pflänzchen mit und solche ohne Mykorrhizen finden. Die ersteren sind schön, die letzteren dagegen im Absterben begriffen." Such a relation might be explained however in just the opposite sense, that is, a considerable number of fresh mycorrhizas should be formed only on roots of vigorous plants as these produce adundant new roots, while feeble ones can afford only a poor development of the mycorrhiza.[1]

As a third evidence he states that "der Verdaung der Hyphen, die in den endophytisch infizierten Mykorrhizen stattfindet, spricht wenigstens für die Wahrscheinlichkeit, dass hier der höhere Symbiont gefördert wird." Indeed, so far as the digestion of the endophytes occurs, the higher

1) This last view has been enforced by my latest excursion to the island of Sakurajima, which remains after the erruption of 1914 still partly bare. Reports will follow in detail when an oppotunity offers itself.

plants may be more or less benefited by it, but as such a case is rather rare in woody plants, it is too far to conclude that the mycorrhiza in general is in a symbiotic association. On the contrary it is purely parasitic or at most only hemi-symbiotic.

VI. CONCLUSION.

The main contributions contained in the present investigation may be summarized as follows:

1. The mycorrhizas of woody plants may be classified into three forms, namely, the ectotrophic, heterotrophic and ecto-endotrophic mycorrhizas. The true heterotrophic mycorrhizas may be rather few, but, among the ectotrophic ones, those which show more or less the heterotrophic nature in old stage of their development are many. These mycorrhizas as well as the heterotrophic ones may be regarded as intermediate between the true ectotrophic and ecto-endotrophic ones.
2. The mycorrhiza-formers may be divided into two groups, namely, the facultative and obligate ones. The former comprises *Boletus bovinus*, *B. luteus (?)*, *Scleroderma vulgare (?)* and *Tricholoma Shimeji*, and the latter *Armillaria caligata*, *A. Matsudake*, *Cantharellus floccosus*, *Cortinarius*, *Hydnum affine* and *Polyporus leucomelas*. Each fungus usually forms a definite mycorrhiza differing from the others, with roots of a definite plant.

   Whether the mycorrhiza becomes ectotrophic, heterotrophic or ecto-endotrophic, is chiefly determined by the specific nature of each fungus concerned. The fate of the intracellular hyphae in the host cells is also destined by the nature of each mycorrhizal fungus. Usually the mycelium of *Boletus* is easily killed and digested by the host cells, while that of *Armillaria*,

Cantharellus, Hydnum and *Polyporus* resists strongly.

3. The mycelial source, from which the infecting hyphae are produced, seems to differ according to the nature of mycorrhiza-formers. In most cases of the obligate mycorrhiza-formers, the infection on young roots may take place by hyphae projected from preexisting mycorrhizas and perhaps by hyphae germinated directly from spores, while in the facultative ones, as they are found in the soil in a saprophitic condition, the infection may be easily carried on also by hyphae existing in soil.
4. In the case of the usual mycorrhizas, the formation of the fungous mantle precedes that of HARTIG's network, while in growing large roots the order is just the reverse.
5. There is a coincidence of seasons in which the mycorrhizas of certain plants become plentiful and in which a great number of new roots are formed, althogh the relation may be more or less modified by some other factors.
6. All the mycorrhizas of woody plants do not show the same biological significance. In the case of the obligate mycorrhiza-formers, such as *Hydnum affine, Cantharellus floccosus, Polyporus leucomelas, Armillaria caligata* and *A. Matsudake*, they not only suck off amino-acids and sugar, from the growing root, but also supply almost no essential salts to them. The fruiting bodies of these fungi are originated moreover chiefly at the expense of roots. No evidence is found that the roots are benefited by association with such fungi. The mycorrhizas caused by such mycorrhiza-formers are, therefore, instances of the parasitism of fungus on the roots of vascular plants.

On the other hand the facultative mycorrhiza-formers are more or less mutual with vascular plants. I mention *Boletí* as a representative of this class, which, on the one hand, supply a certain amount of phosphorus and other salts to the host root, in connection with the mycorrhiza formation, though, on

the other hand, they suck off, amino-acids and sugar, which are to be used for the growth of roots, ultimately causing death. Therefore I may call them briefly "hemisymbionts."

Mycorrhizas caused by *Cortinarius* are considered to be intermediate between the parasitic and the hemi-symbiotic associations, though they seem more or less inclined toward the former side.

VII. SUMMARY.

1. The following fungi have been determined to be mycorrhiza-formers:
   a. *Armillaria caligata* on *Pinus densiflora*.
   b. *A. Matsudake* on *P. densiflora*.
   c. *Boletus bovinus* on *P. densiflora*.
   d. *Cortinarius cinnamomeus* on *Populus tremula var. villosa* and *Pinus densiflora*.
   e. *C. sp. (a)* on *Alnus japonica* and *Castanea pubinervis*.
   f. *C. sp. (b)* on *P. densiflora*.
   g. *C. sp. (k)* on *P. densiflora*.
   h. *Cantharellus floccosus* on *P. densiflora*.
   i. *Hydnum affine* on *P. densiflora*.
   j. *Polyporus leucomelas* on *P. densiflora*.
   k. *Scleroderma vulgare (?)* on *Castanea pubinervis*.
2. The mode of origination of the fruiting bodies of these mycorrhiza-formers may be summarized as follows:
   a. The fruiting body originates directly on an infected root. Example: *Polyporus leucomelas*.
   b. Fruiting bodies originate, without relation with humus, from the mycelium interwoven by the hyphae projected from numerous mycorrhizas. Examples may be found in obligate mycorrhiza-formers such as *Armillaria caligata, A. Matsudake, Hydnum affine, Cantharellus floccosus* and *Polyporus*

leucomelas.

c. Fruiting bodies originate on one, two or more hyphal bundles projected directly from the mycorrhizas, which have connection with humus. Example: *Cortinarius sp. (a)*, *C. sp. (p)*, *C. sp. (k)* and *C. cinnamomeus.*

d. Fruiting bodies usually originate on humus, frequently connected with living roots. Examples may be found in such facultative mycorrhiza-formers as *Boletus bovinus* and *Scleroderma vulgare (?).*

3. Mycorrhizal fungi infect not only side-branches but also growing large roots. In this case, usually heterotrophic mycorrhizas are formed.

4. The following mycorrhizal fungi have been cultivated on an artificial medium:

a. *Armillaria caligata* (from fruiting bodies).

b. *A. Matsudake* (from fruiting bodies and spores).

c. *Boletus bovinus* (from fruiting bodies).

d. *B. luteus (?)* (from mycorrhizas).

e. *Scleroderma vulgare (?)* (from fruiting bodies).

f. *Tricholoma Shimeji* (from fruiting bodies).

5. The following plants gave positive results in the mycorrhiza formation by the synthesis:

Fungi concerned.	Host plants.
Armillaria caligata	*P. densiflora.*
A. Matsudake	*P. densiflora.*
Boletus bovinus	*P. densiflora.*
B. luteus(?)	*P. Tumbergii, Quercus myrsinaefolia, Q. phyllyraeoides, Q. glauca, Q. grosseserrata* and *Q. pausidentata.*
Tricholoma Shimeji	*P. densiflora.*

6. Mycorrhizas resulting from synthesis were generally heterotrophic ones.

7. The period of the fullest development of the mycorrhiza

coincides usually with the period of luxuriant growth of the host root.

8. The growing point of normal roots contains a large amount of amino-acids and sugar which are to be used for its further growth.

9. These nutrients of the root are gradually diminished with the fungous infection, until, in old mycorrhizas, no nutrients are found in the root-tissue.

10. The fact, that the growth of the root is inhibited by the fungous infection, may chiefly be due to the loss of nutrients, because those roots, which retain them still in tolerable amount, can grow further, pushing aside the fungous mantle.

11. All the ectotrophic mycorrizas of woody plants are annual, as stated by McDougall.

12. The facultative mycorrhiza-formers supply after the formation of the mycorrhiza a larger amount of phosphorus to the host plant than the uninfected root can normally absorb, whereas in the obligate ones the relation is just the opposite.

13. In the case of the *Boletus*-mycorrhiza, the roots digest the intracellular hyphae and so derive benefit from the association.

14. In the obligate mycorrhiza formers, their fruiting bodies are formed chiefly at the expense of the roots of host plants.

15. Mycorrhizas caused by many obligate mycorrhiza-formers are considered to be instances of parasitic associations of fungi on roots.

16. The facultative mycorrhiza-formers such as *Bolcti* are considered to be hemi-symbionts of woody plants.

17. *Cortinarius*-mycorrhizas may be intermediate between the parasitic and hemi-symbiotic associations, although they are considered to be more or less inclined towards the parasitic side.

In conclusion I wish to express sincere thanks for many helpful suggestions to Professor K. Kôriba, under whose direction this study was undertaken.

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EXPLANATION OF PLATES.

Plate VII.

Figs. 1—6. Cells of an uninfected root obtained from pines cultivated on sterilized soil in ERLENMEYER-flasks. ×1300.

1, A meristematic cell; 2—4, young cells of the periblem; 5—6, fully-developed cells of the cortex. *n*, nucleus; *t*, tannic granule; *v*, vacuole.

Figs. 7—13. Cluster-type mycorrhiza. 8—13, ×1300.

7, Median longitudinal section of the mycorrhiza; 8, cells and degenerated cells of the meristematic portion; 9, a young cell of the periblem; 10—11, fully-developed cells of the cortex; 12—13, nuclei of fully-developed cortical cells; *c*, cortex; *cc*, central cylinder; *d*, degenerated cell; *f*, fungous mantle; *H. n.*, HARTIG's network; *n*, nucleus; *t*, tannic granule; *v.m.*, vacuolar membrane.

Plate VIII.

Fig. 1. Young fruiting bodies of *Armillaria caligata* originated on a mycelial mass. *F*, Young button; *M*, mycorrhizal mass. ×1.

Fig. 2. *Boletus bovinus* originated in connection with mycorrhizas of *Pinus densiflora*. × ca 1.

Fig. 3. Hyphal bundles of *Scleroderma vulgare (?)* attached to mycorrhizas of *Castanea pubinervis*. *F*. Basal portion of the fruiting body; *M*, mycorrhizas.

Fig. 4. Young fruiting bodies, *F*, of *Cortinarius cinnamomeus* originated on hyphal bundles produced from mycorrhizas of *Pinus densiflora*, *M*. × ca 2.

Plate IX.

Fig. 1. *Polyporus leucomelas*, *F*, originated directly on an infected root of *Pinus densiflora*, *R*. × ca 1·5.

Fig. 2. Young fruiting body of *Cortinarius sp. (?)*, *F*, formed at the termination of a hyphal bundle, *H*, produced by mycorrhizas of *Pinus densiflora*, *M*.

Fig. 3. Several buttons, ×, of *Armillaria caligata* originated on a mycelium formed by numerous hyphae projected from many mycorrhizas of *Pinus densiflora*.

Fig. 4. Vertical section of a mycorrhizal layer, *M*, and submycorrhizal one, *S*, of *Hydnum affine*. × ca 2·5.

Fig. 5. Young buttons, *F*, of *Armillaria Matsudake* produced on a mycorrhizal mass, *M*, formed by numerous hyphae projected from abundant mycorrhizas of *Pinus densiflora*. × ca 2·5.

Plate X.

Fig. 1. Mycorrhizas of *Pinus densiflora* caused by *Armillaria caligata*. × ca 3.

Fig. 2. Mycorrhizas of *P. densiflora* caused by *Cortinarius sp. (p)*. × ca 2·5.

Fig. 3. A large root of *Populus tremula var. villosa* infected by the mycelium of a mycorrhiza in contact. ×3·5.

Fig. 4. Mycorrhizas of *Populus tremula var. villosa* caused by *C. cinnamomeus*. ×7.

Fig. 5. Large root of *Populus tremula var. villosa* half-enveloped by the fungous mantle. ×3·5.

Fig. 6. Two buttons, *B*, originated on the mycorrhizas of *Pinus densiflora* caused by *Cortinarius sp. (k)*, *M*. ×3·6.

Fig. 7. Mycorrhizas of *Pinus densiflora* caused by *Cortinarius cinnamomeus*. ×2·5.

Fig. 8. Mycorrhizas of *Pinus densiflora* caused by *Cantharellus floccosus*. ×1·6.

Fig. 9. Roots of *Pinus densiflora* infected by a mycelium of *Polyporus leucomelas*. × ca 2.

Plate XI.

Fig. 1. Mycelium of *Armillaria caligata* in pure culture. *A*, Mycelium grown on starch-agar; *B*, on meat–extract–malt–extract agar. ×1.

Fig. 2. Mycelium of *Boletus bovinus* in pure culture.× 1·2.

Fig. 3. Mycelium of *Armillaria caligata* in pure culture. ×200.

Fig. 4. Mycelium of *Tricholoma Shimeji* in pure culture. ×1·5.

Fig. 5. Mycelium of *Boletus luteus* (?) in pure culture. ×$\frac{3}{4}$.

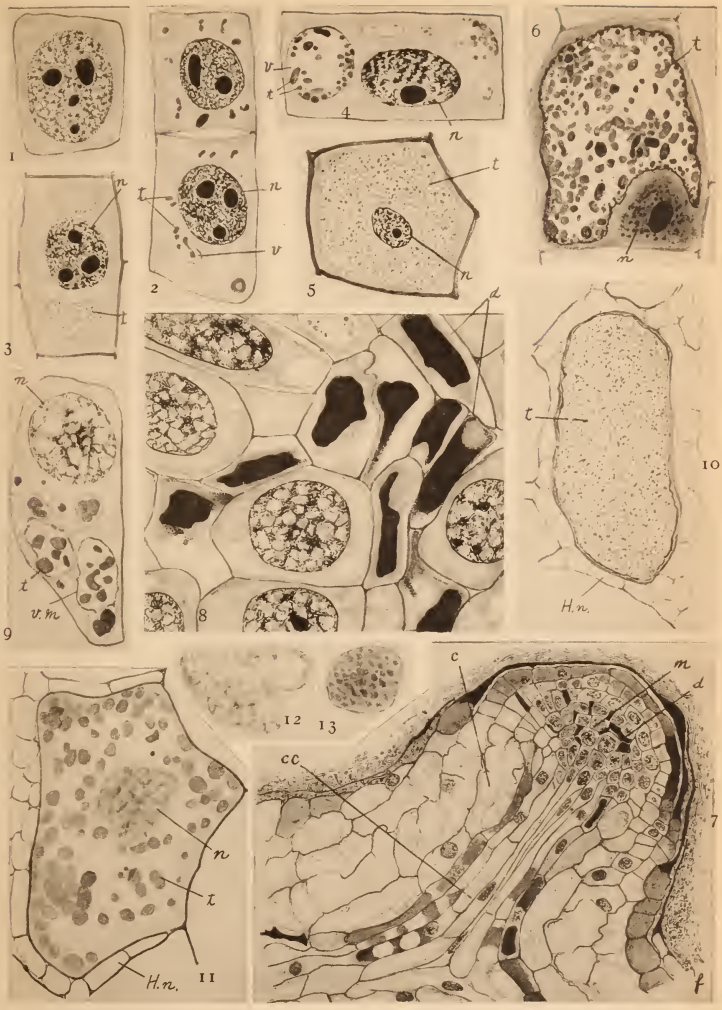
Fig. 6. Mycelium of *Armillaria Matsudake*. ×1.

Fig. 7. *Pinus densiflora* and *Boletus bovinus* cultivated in a flask, showing mycelium, *M*, developed along the stems. × ca $\frac{1}{2}$.

Fig. 8. Old mycelium of *Boletus bovinus* in pure culture, showing locally swollen cell-wall. ×500.

Fig. 9. A magnified root of a pine infected by *Armillaria Matsudake* in synthesis.

Fig. 10. Mycorrhizas of *Pinus Tumbergii* caused by *Boletus luteus* (?) in synthesis. ×6.



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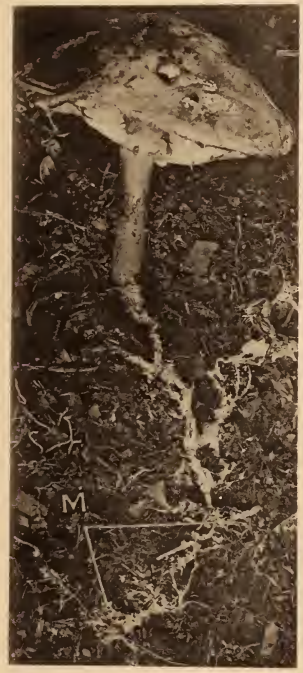

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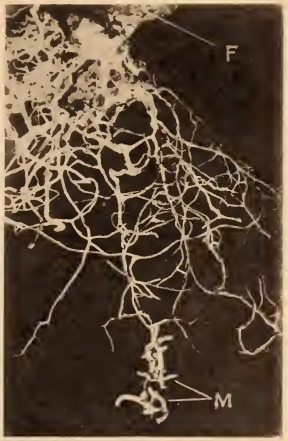

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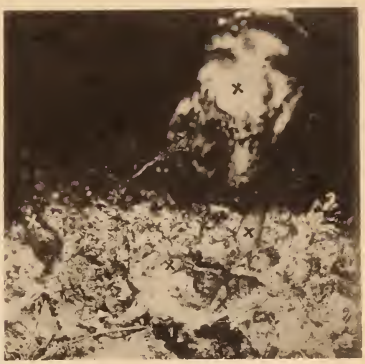

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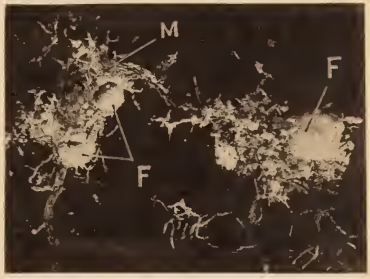

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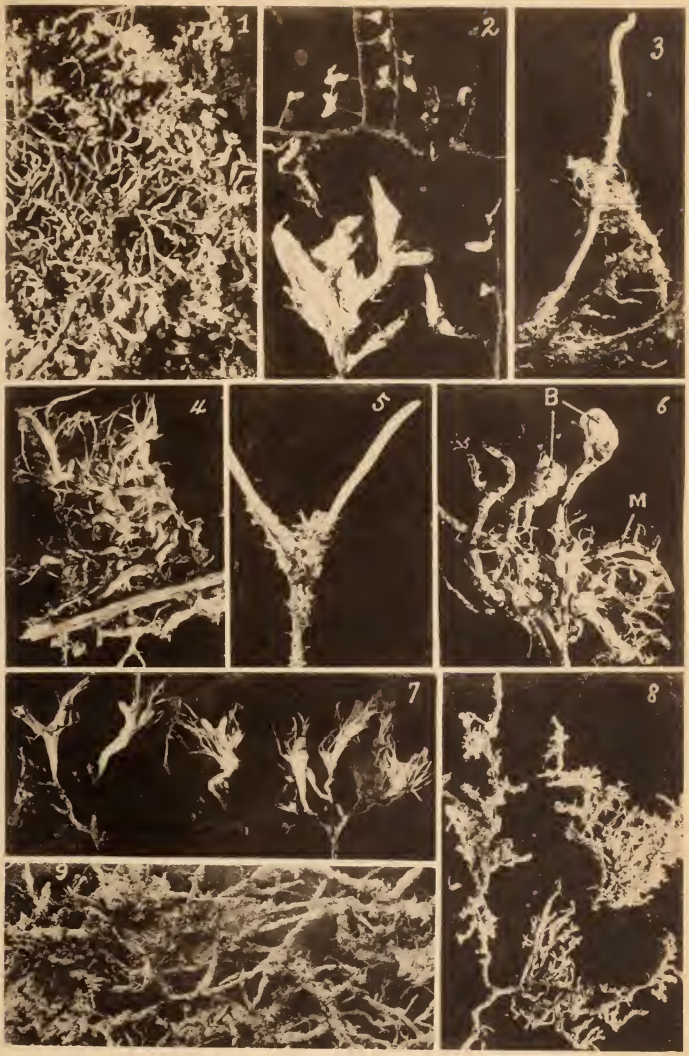

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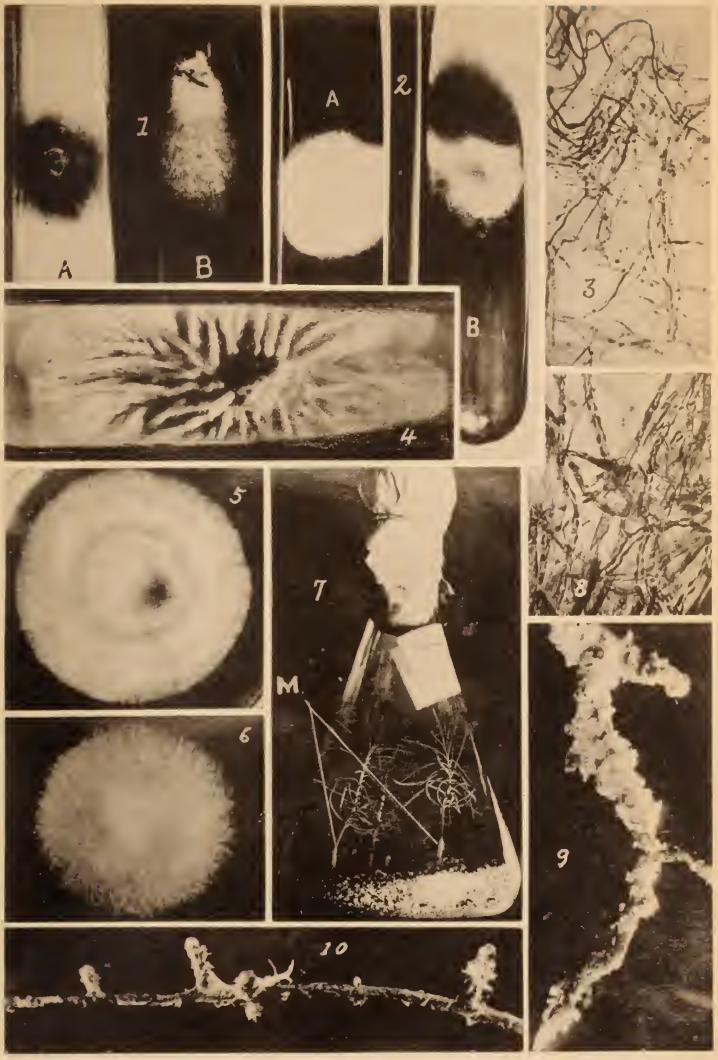

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